

96/34888 (November 7, 1996) and (2) demonstrate diligence starting prior to these effective dates and continuing to the inventors' constructive reduction to practice of the invention.

Applicants now enclose their Rule 131 declaration for the Examiner's full consideration. Attached to the inventors' declaration are Exhibits 1 and 2. Exhibit 1 is a conception record for applicants' invention. Exhibit 2 is a supporting declaration of Dr. Krul demonstrating the inventors' diligence.

As noted in the inventors' Rule 131 declaration, they conceived the invention prior to November 7, 1996, *i.e.*, prior to the effective dates of both WO 96/39168 (December 12, 1996) and WO 96/34888 (November 7, 1996). The actual dates have been redacted from the submitted conception record. The submitted conception record shows that the inventors had a conception of the complete and operative invention of using a peptide as an immunogen, such as the C-terminal 26 amino acids of CETP conjugated to a carrier protein such as thyroglobulin or Keyhole Limpet hemocyanin, for generating an autoimmune response that neutralizes endogenous CETP. Booster injections would be given periodically after the initial immunization to generate a high titer antibody response against the peptide, which antibodies would also recognize intact endogenous plasma CETP.

As noted in the inventors' Rule 131 declaration, they also were diligent in seeking to reduce their invention to practice from a date starting prior to November 7, 1996, *i.e.*, prior to the effective dates of both WO 96/39168 (December 12, 1996) and WO 96/34888 (November 7, 1996) and proceeding continuously until the constructive reduction to practice of the application by their filing the original application on January 21, 1997. The pending application is a Continued Prosecution Application of a continuation of this original application.

The inventors' diligence is established by Exhibit 2, a declaration of Dr. Elanie Krul, which declaration is attached to the inventors' declaration.

Dr. Krul, who is not an inventor of the invention defined by the pending claims, testifies, that both she and individuals working under her direction and control, worked every day, save for weekends and Searle holidays, in an effort to reduce the invention to practice on behalf of the inventors. The work continued uninterrupted from a date starting prior to the effective dates of both WO 96/39168 (December 12, 1996) and WO 96/34888 (November 7, 1996) and proceeding through a date subsequent to the January 21, 1997 constructive reduction to practice. Dr. Krul's declaration has attached as Exhibit A the relevant pages of notebook records memorializing the work that was done during the recited time frame. Examiner Davis was shown Exhibit A during the interview. Applicants would also like to repeat the offer made at the interview to provide whatever assistance she requires to understand the contents of Exhibit A.

Applicants submit that Exhibits 1 and 2, when considered with their Rule 131 declaration, establishes prior conception and diligence to the constructive reduction to practice of their invention. As a result, neither WO 96/39168, nor WO 96/34888 can properly be cited against the pending claims.

During the interview, Examiner Davis also alluded to her desire to have explained on the record the patentable distinction between the claimed invention and the subject matter of several documents previously cited in submitted information disclosure statements, viz, [A19] Swenson et al. J. Biol. Chem., 264:14318-14326, 1989; [A21] Whitlock et al. J. Clin. Invest., 84:129-137, 1989; [A22] Evans et al. J. Lipid Res 35:1634-1645, 1994; and [A23] Zuckerman et al. Lipids, 30:307-311, 1995.

As explained to Examiner Davis at the interview, each of these documents was identified and distinguished within the body of the pending application. Each of these articles is directed to passive xenogenic immunization and thus differs radically from the active autogenic immunization of the pending claims.

The cited articles simply chronicle the temporary reductions in CETP that might be accomplished using passive immunization with xenogenic antibodies (a “process in which antibodies [produced] from an animal of one species are administered to an animal of another species, [see application page 13 ln 31-33]). The following examples of passive xenogenic immunizations from these documents are thus specifically discussed/distinguished in the subject application:

- Monoclonal antibody TP2 was produced by hybridoma technology (using xenogenic human CETP). “TP2 is directed against an epitope within the last 26 amino acids of CETP (SEQ ID NO:29) . . . [and] has been shown to block CETP-mediated lipid transfer . . .” (page 6, ln 7-15). [A19] Swenson et al. J. Biol. Chem., 264:14318-14326, 1989.

- “[R]abbits were intravenously injected with TP1 [xenogeneic mouse monoclonal antibodies], resulting in an initial 70 percent inhibition of CETP-mediated CE transfer activity. (Page 6, ln 16-21). [A21] Whitlock et al. J. Clin. Invest., 84:129-137, 1989

- “[A] single sub-cutaneous injection of TP2 monoclonal antibodies in another illustration of passive administration of xenogeneic antibodies [into hamster] . . . raised HDL cholesterol 24 percent. [page 6, ln 33 through page 7, ln 6] [A22] . Evans et al. J. Lipid Res 35:1634-1645, 1994; [A23] Zuckerman et al. Lipids, 30:307-311, 1995.

As discussed during the interview, notable limitations of passive xenogenic immunization disclosed in the subject application include the fact that:

- “Passive immunization with the use of xenogeneic antibodies can only be utilized for a short-term period of time because host animals develop antibodies to the xenogeneic immunoglobulin.” [pg 8, ln 18-21] and

- The need for, and “problems associated with [frequently] repeated administration. [page

Passive xenogenic immunizations can be readily distinguished from the patentably distinct active autogenic immunization defined by the pending application and claims as set forth in the Table below:

	SOURCE OF ANTIBODIES	CETP	INJECTANT	FREQUENCY OF INJECTION (TO MAINTAIN ELEVATED HDL-CHOLESTEROL)	SIDE EFFECTS	EXAMPLES
PASSIVE XENOGENIC IMMUNIZATIONS	Produced by donor animal	Xenogenic to host	Antibodies against CETP	Every few days	Anaphylaxis, elicits antibody response to injected antibodies	A19 Swenson et al. A21 Whitlock et al. A22 Evans et al. A23 Zuckerman et al.
ACTIVE AUTOGENIC IMMUNIZATION	Endogenousl y produced by treated subject	Autogenic to host	CETP	Every 9-18 months	No anti-antibody response (antibodies not injected)	Present invention

As examiner Davis herself observed at the interview, the success of autogenic immunization in view of a prior disclosure of passive xenogenic immunization would not have been expected because of mechanisms normally leading to self tolerance (e.g. central T cell tolerance, peripheral T

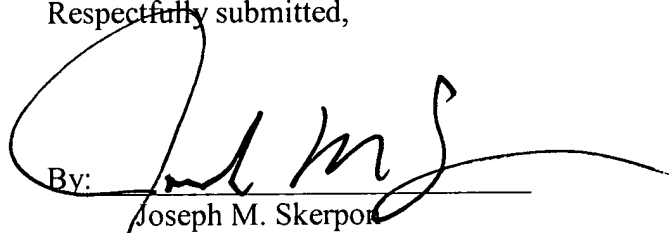
cell tolerance, T cell anergy, etc.) (Cellular and Molecular Immunology, 4th ed. Eds. Abbas, Lichtman, and Pober, Saunders Company, 2000). Moreover, in view of, for example, the work of L. Sherman and others, the action of CTLA-4 would be expected to prevent antibody production against "self" (autogenic) proteins (J Immunol. 2001 Mar 15; 166(6):3908-14).

On the basis of the above, applicants respectfully request consideration of the subject application.

Please charge our Deposit Account No. 19-0733 for any fee.

Respectfully submitted,

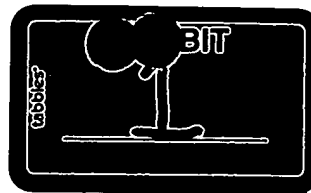
By:


Joseph M. Skerpton
Registration No. 29,864

Dated: April 18, 2002

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1001 G Street, N.W., Eleventh Floor
Washington, D.C. 20001-4597
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COMPANY CONFIDENTIAL



P9331

DISCLOSURE OF INVENTION
MONSANTO CORPORATE RESEARCH

DISCLOSURE NO.
D-07-

This disclosure has been read and is understood by me:

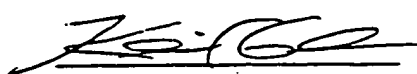
ROUTE (as appropriate):

SIGNATURE

DATE

Dr. Kevin C. Glenn

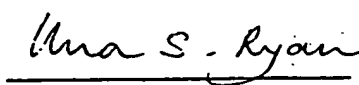
Associate Fellow






Dr. Una S. Ryan

Research Director





Dr. Philip Needleman

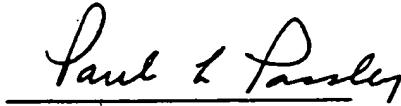
Corp. Vice President





Paul Passley

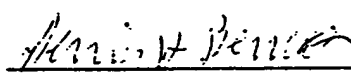
Patent Counsel





Dennis Bennett

Attorney





1. SUGGESTED TITLE:

Improvement of Plasma HDL and LDL Cholesterol Levels By Generation of Autoimmune Neutralization of Plasma Cholesteryl Ester Transfer Protein (CETP)


2. (a) Give a short statement of invention.

Peptide or peptido-mimetic immunogens are used to generate a neutralizing autoimmune reaction to endogenous plasma CETP that significantly elevates plasma HDL cholesterol and lowers LDL cholesterol sufficient to markedly reduce an individual's risk of coronary artery disease.

(b) Describe the invention in a broad manner and give at least one complete example. (Use additional sheets if necessary.)*

Mortality statistics for the past several decades demonstrate an "epidemic" rise in the incidence of coronary artery disease (CAD) in Western industrialized countries, with a sizable number directly linked to disturbances in blood lipoprotein levels. Numerous

*Attach, sign and date all additional sheets.


Signature/Date



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drugs are available for treating elevated plasma low density lipoprotein cholesterol (LDLc) levels. However, several recent clinical studies have established an inverse relationship between plasma high density lipoprotein cholesterol (HDLc) and the incidence of (CAD), suggesting that increased HDLc may protect or even reverse coronary disease related to high LDLc.

It has recently been shown that the presence and level of cholesteryl ester transfer protein (CETP) in the plasma correlates with elevated LDLc and lowered HDLc. CETP's normal function is to transfer cholesteryl ester from HDL to LDL. Individuals genetically deficient in CETP have remarkably high HDLc and low LDLc, and appear to live normal healthy lives while enjoying remarkable longevity into their 80's and 90's. CETP is a 74,000 dalton acidic glycoprotein. Deletion of the C-terminus of CETP renders it inactive for promoting lipid transfer between HDL and LDL. Site-directed mutagenesis and a monoclonal antibody that blocks CETP action have shown that the C-terminal 26 amino acids are essential for binding of CETP to LDL and HDL for lipid transfer. An effective inhibitor of CETP could represent a breakthrough product that could supplant current therapies focused on improving an individual's plasma cholesterol profile.

The current invention involves the use of a peptide or peptido-mimetic as an immunogen for generating an autoimmune response that neutralizes endogenous CETP. Central to the invention is the concept that auto-immunologic inhibition of CETP will result in marked elevation of HDLc and reduced LDLc, similar to that seen in individuals that are genetically deficient in CETP. The peptide or peptido-mimetic immunogens could represent all or part of the CETP protein molecule, including but not restricted to the C-terminal 26 amino acid region of CETP shown to account for CETP-facilitated lipid transfer activity.

An example would be the use of a chemically synthesized peptide of 26 amino acids in length with the sequence:

RDGFLLLQMDFGFPEHLLVDFLQSL

representing the C-terminal 26 amino acids of human CETP. This peptide would be conjugated to a carrier protein, such as thyroglobulin or Keyhole Limpet hemocyanin (KLH), and injected subcutaneously with an adjuvant. Booster injections would be given 4 to 6 weeks after the initial immunization and every 3 to 4 weeks subsequently, in order to generate a high titer response against the peptide that will also recognize intact endogenous plasma CETP. Plasma HDLc and LDLc is measured before and after immunizations to monitor efficacy of the method towards improving an individual's lipoprotein levels such that they have a reduced risk of CAD.

(c) List variables and alternatives which can be used.

The 26 amino acid C-terminal peptide of CETP is so hydrophobic that it may be able to serve as a suitable immunogen without the need for conjugation to a carrier protein. Other regions of CETP, or the full length protein may also be suitable immunogens for generating an autoimmune neutralizing antiserum against CETP activity in the plasma. It would be valuable to use peptides smaller than the full-length CETP or even regions of CETP such as its 26 amino acid C-terminal region to generate a neutralizing immunological response to endogenous CETP. In fact, a peptide may not be necessary,

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but instead a peptido-mimetic that is functionally equivalent to the critical region of CETP required to generate antibodies that neutralize endogenous CETP but that chemically is more stable or immunogenic than the native peptide chemical structure. Substitution of amino acids unique to human CETP with the analogous amino acid from another species may enhance generation of neutralizing antibodies. Alternatively, non-natural amino acids (e.g. D versus L-form) at specific places within the immunogen may facilitate generation of high potency autoimmune-generated neutralizing antibodies.

In place of synthetically generated peptide immunogen, the pGEX system of producing a fusion protein between CETP or CETP-derived peptide and glutathione S-transferase protein (GST) in *E. coli* could present a viable immunogen for generating autoimmune antibodies that neutralize endogenous CETP activity.

The adjuvant vehicle and route of immunization is another set of variables that could influence the probability of generating a neutralizing autoimmune reaction to endogenous CETP. Several types of adjuvant are possible: Complete and Incomplete Freund's adjuvant, synthetic adjuvants (such as muramyl dipeptide derivatives sold by Ribi Immunochem Research, Inc, Hamilton MT or TiterMax sold by CytRx Corp., Norcross, GA), and/or oil emulsions. Alternatives to subcutaneous injections include transdermal, intramuscular, or intravenous injection or combinations of the above. Another option is to alternate the carrier protein for different injections. For example, the first injection could be with the peptide conjugated to Carrier "A" (e.g. thyroglobulin or GST) and booster injections could be with Carrier "B" (e.g. KLH or peptide alone).

- (d) How does this differ from the previous work of others. Give citations and attach references (if available).

To date, the only published attempt to neutralize CETP action has been with a mouse monoclonal antibody that blocks CETP-mediated lipid transfer *in vitro* (Hesler, C.B., Tall, A.R., Swenson, T.L., Weech, P.K., Marcel, Y.L. and Milne, R.W. (1988) *J. Biol. Chem.* 263: 5020-5023, Swenson, M.J., Hesler, C.B., Brown, M.L., Quinte, E., Trotta, P.P., Haslanger, M.F., Gaeta, F.C.A., Marcel, Y.L., Milne, R.W., and Tall, A.R. (1989) *J. Biol. Chem.* 264: 14318-14326). Passive immunization of rabbits with the blocking monoclonal antibody produced a modest decrease in plasma LDLc (~20%) and modest increase in plasma HDLc (~10%) (Whitlock, M.E., Swenson, T.L., Romakrishnan, R., Leonard, M.T., Marcel, Y.L., Milne, R.W. and Tall, A.R. (1989) *J. Clin. Invest.* 84: 129-137). The marginal effect of the monoclonal antibody could be owing to 1) the short duration of the passive immunization protocol (7 days), 2) the generation of endogenous anti-rabbit antibodies that impaired passive antibody activity, 3) less than complete inhibition of endogenous CETP by inappropriate dosage of the blocking monoclonal antibody, or 4) their showing that TP2 could inhibit rabbit CETP approximately 62%. The present invention would involve generation of an autoimmune response to endogenous CETP that would be a significant improvement over the previous work by others by avoiding the complicating issues listed above.

3. (a) When was the invention first thought of?

The invention was first developed at a meeting with Dr. Phillip Needleman at a meeting held [REDACTED] Contributors to the initial conceptualization of the invention of

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autoimmune neutralization of endogenous CETP as a way of improving an individual's plasma HDL and LDL profile included Dr's: Needleman, Ryan and Glenn.

- (b) Give date of the first written description (include notebook page numbers).

The first written documentation of the invention was a printout of a ccMAIL message from Dr. Needleman dated [REDACTED] and is included as an attachment to notebook page [REDACTED] as a part of Dr. Glenn's complete description of the invention on that page, dated [REDACTED]

4. To whom and on what date did you first disclose or suggest this invention to others, either orally or in writing?

The people present at the meeting held [REDACTED] included: Dr. Needleman, Dr. Una Ryan, Dr. Donald Laird, Michele Melton, Dianne Stockhausen and myself. Since the time of that meeting, the invention has also been discussed with other Monsanto or Searle employees including: Dr. Gwen Krivi, Dr. Robert Manning, Dr. Ben Schwartz, Dr. Dan Connolly, Tim Keane, and Dennis Bennett, and presented at the Vascular Biology Targets meeting [REDACTED] It has never been disclosed outside Monsanto/Searle, either orally or in writing.

5. Give dates and details regarding samples, information, or publication, relating to this invention which have been or are currently planned to be given to persons outside Monsanto.

Currently, no plans exist for providing samples, information or publication of information.

Signature: [Signature] Date: [REDACTED]

Print Name: Kevin C. Glenn
First Middle Last

Signature: Una S. Ryan Date: [REDACTED]

Print Name: UNA S. RYAN
First Middle Last

Signature: [Signature] Date: [REDACTED]

Print Name: Philip - NEEDLEMAN
First Middle Last

[Signature] [REDACTED]
Signature/Date



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application Of:)	
)	
Needleman et al.)	Group Art Unit 1642
)	
Serial No. 09/387,340)	Examiner: Minh-Tam Davis
)	
Filed: August 31, 1999)	Atty. Docket: MON-102.0-C3119-C
)	061765.00367
Continued Prosecution Application)	
Filed: January 4, 2002)	
)	
)	

For: AN IMMUNOLOGICAL PROCESS FOR INCREASING HDL CHOLESTEROL
CONCENTRATION (AS AMENDED)

DECLARATION OF ELAINE KRUL PURSUANT TO 37 C.F.R. §1.131

The Honorable Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, ELAINE KRUL, Ph.D. hereby declare that:

1. I am presently employed by Pharmacia Corporation.
2. I received my Ph.D. degree from McGill University, Montreal, Quebec, Canada in 1982, majoring in biochemistry;
3. Between February of 1994 and February of 1998, I was employed by G.D. Searle, Co., ("SEARLE"), now a wholly owned subsidiary of Pharmacia. Pharmacia, formerly known as Monsanto Company, is the assignee of the above-identified patent application.
4. The work described in this Declaration was carried out in the State of Missouri, the United States of America, by me or by one or more persons under my direction and control and the work described in this declaration was done on behalf of the inventors of the above-identified patent application.

5. The work described in this Declaration occurred during an interval ("INTERVAL") beginning on a date prior to November 7, 1996 (the date of publication of WO 96/34888) and continuing to a date subsequent to January 21, 1997 (the date of filing of U.S. Patent Application Serial No. 08/788,882 for which priority of the above-identified patent application is claimed).

6. During the INTERVAL, such work was conducted towards reducing to practice a vaccination method using an inoculum comprising a vaccine construct for treating human patients to produce antibodies against endogenous CETP such as for the purpose of treating human pro-atherogenic dyslipoproteinemias (e.g., atherosclerosis).

7. Such work comprised (1) vaccinating laboratory animals with inocula, each inoculum containing a vehicle and a CETP construct (CETP immunogen), wherein such constructs consisted of either tuberculin purified protein derivative (PPD) or ~~multiple antigenic peptide (MAP)~~ as an exogenous antigenic carrier polypeptide peptide-bonded to amino acids 42-61, 150-169, 306-325, 345-364, 370-389, or 475-496 of the human CETP amino acid sequence, (2) analyzing the time course of the resultant lipoprotein and cholesterol levels, anti-CETP antibody produced, and endogenous CETP activity levels in the vaccinated animals, and (3) developing a new transgenic mouse model for atherosclerosis to serve as an alternative confirmatory platform to the established rabbit model for testing and validating such vaccination method.

8. As many as five full-time SEARLE employees contributed to the work under my direction.

9. Excepting weekends and SEARLE holidays, such work was conducted every day during the INTERVAL.

10. I make the foregoing statements regarding such work after a review of several laboratory notebooks, such notebooks each being bound and paginated and each page being signed and dated by me or by one of the five full-time SEARLE employees working under my direction, or control. True copies of the relevant pages of the notebook records are appended hereto as Exhibit A

11. I am over 18 years of age and of competent mind.

12. All statements made of my own knowledge are true and all statements made on information and belief are believed to be true; and further, these statements were made with the knowledge that willful, false statement so made are punishable by fine or imprisonment or both, under 18 U.S.C. § 1001 and that such willful, false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

4/17/02

Date

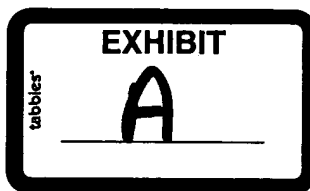
Elaine Krul

Elaine Krul

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Project Number	Subject	Book Number
SEARLE	Terminal Boosts & Bleeds of Rabbit 02, 07 & 08	GDS - 5734
		Page 053

Rabbit 02, 07, 08 boosted on 10-12-96 with
OETP-peptides coupled to PPD. (see notebook 5734001-007)
(Harriet Kuulander prepared emulsions) (Rabbit 01 sacrificed
several months ago due to infection in eye.)
Rabbit bled out on 10-25-96. Sera frozen & stored
at -20°C.



PPD = tuberculin
purified protein derivative.

-1-97

Author's Signature	Date	Read and Understood By	Date
Elaine S. Kuhl	10/26/96	Denise Nachowiak	10-1-97

Book Number GDS - 5734	Subject CETP associated IgM: Binding to oxLCL	Project Number SEARLE
Page 054		

Hypothesis: CETP assoc. IgM is binding oxLCL lipids on CETP (antibody to oxLCL)
CETP prep from Heidi Hoyer (6/12/96) was applied
(by Heidi) onto Pierce IgM column

We obtained 8.7 ml of eluted protein
in the Pierce elution Buffer (~20 µg/ml by
A₂₈₀ nm)

I added 8.7 ml saturated (NH₄)₂SO₄ dispense
let sit for several days @ 4°C.

Spun @ 10,000 rpm in JA rotor (7/19/96).
Could not see precipitate - saw "cloudy"
material on wall of tube - Dissolved in
200 µl PBS.

Performed downy. (see p.056)

Obtained LCL₁₄₇ from Annette Frick.

Oxidized LCL₁₄₇ according to protocol on opposite
page.

Performed downy. (see p.056)

Author's Signature <i>Elaine Krul</i>	Date <i>10/26/96</i>	Read and Understood By <i>Denise Nachowiak</i>	Date <i>10-1-97</i>
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Project Number	Subject	Book Number
SEARLE	CETP associated IgM: Binding to oxLDL	GDS - 5734
		Page 055

CU²⁺ OXIDATION OF LDL

Refs: 1) Steinbrecher, U.P., Witztum, J.L., Parthasarathy, S. and Steinberg, D. (1987) Decrease in reactive amino groups during oxidation or endothelial cell modification of LDL, Arteriosclerosis 7: 135-143.

2) Barnhart, R.L., Busch, S.J. and Jackson, R.L. (1989) Concentration-dependent antioxidant activity of probucol in low density lipoproteins in vitro: probucol degradation precedes lipoprotein oxidation, J. Lipid Res. 30: 1703-1710.

Materials

- 1 mM CuSO₄•5H₂O (0.025g QS to 100 ml)
- EDTA-free PBS (regular PBS)
- LDL (at least a 2 ml solution diluted to 300 µg/ml protein with PBS)
- Buffer A - 0.01 M sodium phosphate, 0.15 M NaCl, 0.01% EDTA, pH 7.4
1.56 g NaH₂PO₄•H₂O
4.07 g Na₂HPO₄
8.8 g NaCl
0.1 g EDTA (or 0.54 ml of a 0.5 M EDTA STOCK, pH 8)
Bring up in approx. 900 ml of deionized H₂O. Adjust pH to 7.4 and QS to 1 liter.

Procedure

1. Dialyze LDL versus EDTA-free PBS to remove any EDTA or DTPA. (These chelating agents will block the interaction of Cu²⁺ with LDL).
2. Dilute dialyzed LDL solution to a concentration of 300 µg/ml with PBS and put LDL solution into a clean screw-cap tube.
3. Add 1 mM CuSO₄ to LDL solution to a final concentration of 10 µM (1:100 dilution).
4. Incubate LDL-CuSO₄ mixture at 37°C in loosely capped tube overnight.
5. Terminate reaction by dialyzing LDL solution against Buffer A at 4°C overnight.

A:CUOXLDL.EK
02-21-90

Author's Signature	Date	Read and Understood By	Date
Elaine Kue	10/26/96	Denise Nachowiak	10-1-97

Book Number GDS - 5734	Subject CETP associated IgM: Binding to oxLDL	Project Number SEARLE
Page 056		

	1	2	3	4	5	6	7	8	9	10	11	12
A	-0.001	0.001	0.274	0.243	0.178	0.181	-0.034	-0.035	-0.035	-0.034	-0.035	-0.036
B	0.072	0.074	0.446	0.455	0.020	0.023	-0.034	-0.035	-0.035	-0.034	-0.035	-0.032
C	0.120	0.110	0.598	0.601	0.028	0.034	-0.034	-0.035	-0.035	-0.034	-0.034	-0.027
D	0.201	0.200	0.251	0.237	0.000	0.001	-0.022	-0.031	-0.035	-0.034	-0.034	-0.031
E	0.357	0.365	0.414	0.412	0.011	0.014	-0.021	-0.034	-0.035	-0.034	-0.034	-0.033
F	0.503	0.493	0.558	0.552	0.000	0.001	-0.032	-0.035	-0.035	-0.034	-0.034	-0.034
G	0.683	0.688	0.134	0.133	0.000	0.000	-0.033	-0.035	-0.035	-0.034	-0.032	-0.025
H	0.909	0.863	0.167	0.170	-0.002	-0.001	-0.033	-0.035	-0.035	-0.034	-0.034	-0.022

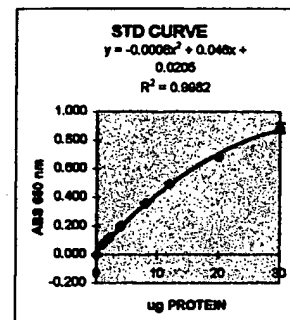
READ DATE:
7/23/98
ASSAY NAME:
PLATE NUMBER:
072396p1
READER NUMBER:

MARKWELL PROTEIN ASSAY

ug	OD 1	OD 2	MEAN	SD			CALC
STD	OD	OD	OD	OD	m	b	STD
0	-0.001	0.001	0.000	0.001			-0.480
1	0.072	0.074	0.073	0.001			1.058
2	0.120	0.110	0.115	0.007			1.971
4	0.201	0.200	0.201	0.001			3.602
8	0.357	0.365	0.361	0.008			7.841
12	0.503	0.493	0.498	0.007			11.634
20	0.683	0.688	0.686	0.004			17.863
30	0.909	0.863	0.886	0.033			27.846

SAMPLE RESULTS (DUPLICATES)

SAMP. NO.	(ml)	OD 1	OD 2	MEAN	SD	CALC. ug	CALC. ug/ml	DF	mg/ml
LDL147	0.010	0.274	0.243	0.259	0.022	5.274	527.380	10.000	5.274
LDL147	0.020	0.446	0.455	0.451	0.008	10.264	513.187	10.000	5.132
LDL147	0.030	0.598	0.601	0.600	0.002	14.813	493.760	10.000	4.938
LDL148	0.010	0.251	0.237	0.244	0.010	4.826	482.592	10.000	4.826
LDL148	0.020	0.414	0.412	0.413	0.001	9.225	461.257	10.000	4.613
LDL148	0.030	0.556	0.552	0.554	0.003	13.341	444.689	10.000	4.447
oxLDL	0.010	0.134	0.133	0.134	0.001	2.381	238.077	1.000	0.238
oxLDL	0.020	0.167	0.170	0.169	0.002	3.168	158.390	1.000	0.158
oxLDL	0.030	0.178	0.161	0.169	0.011	3.168	105.593	1.000	0.106
IgM	0.010	0.020	0.023	0.022	0.002	-0.033	-3.346	1.000	-0.003
IgM	0.020	0.028	0.034	0.031	0.004	0.188	8.281	1.000	0.008
12	0.020	0.000	0.001	0.001	0.001	#DIV/0!	#DIV/0!	1.000	#DIV/0!
13	0.020	0.011	0.014	0.013	0.002	#DIV/0!	#DIV/0!	1.000	#DIV/0!
14	0.020	0.000	0.001	0.001	0.001	#DIV/0!	#DIV/0!	1.000	#DIV/0!
15	0.020	0.000	0.000	0.000	0.000	#DIV/0!	#DIV/0!	1.000	#DIV/0!
16	0.020	-0.002	-0.001	-0.002	0.001	#DIV/0!	#DIV/0!	1.000	#DIV/0!
17	0.020	-0.034	-0.035	-0.035	0.001	#DIV/0!	#DIV/0!	1.000	#DIV/0!
18	0.020	-0.034	-0.035	-0.035	0.001	#DIV/0!	#DIV/0!	1.000	#DIV/0!
19	0.020	-0.034	-0.035	-0.035	0.001	#DIV/0!	#DIV/0!	1.000	#DIV/0!
20	0.020	-0.022	-0.031	-0.027	0.008	#DIV/0!	#DIV/0!	1.000	#DIV/0!
21	0.020	-0.021	-0.034	-0.028	0.009	#DIV/0!	#DIV/0!	1.000	#DIV/0!
22	0.020	-0.032	-0.035	-0.034	0.002	#DIV/0!	#DIV/0!	1.000	#DIV/0!
23	0.020	-0.033	-0.035	-0.034	0.001	#DIV/0!	#DIV/0!	1.000	#DIV/0!
24	0.020	-0.033	-0.035	-0.034	0.001	#DIV/0!	#DIV/0!	1.000	#DIV/0!
25	0.020	-0.035	-0.034	-0.035	0.001	#DIV/0!	#DIV/0!	1.000	#DIV/0!
26	0.020	-0.035	-0.034	-0.035	0.001	#DIV/0!	#DIV/0!	1.000	#DIV/0!
27	0.020	-0.035	-0.034	-0.035	0.001	#DIV/0!	#DIV/0!	1.000	#DIV/0!
28	0.020	-0.035	-0.034	-0.035	0.001	#DIV/0!	#DIV/0!	1.000	#DIV/0!
29	0.020	-0.035	-0.034	-0.035	0.001	#DIV/0!	#DIV/0!	1.000	#DIV/0!
30	0.020	-0.035	-0.034	-0.035	0.001	#DIV/0!	#DIV/0!	1.000	#DIV/0!
31	0.020	-0.035	-0.034	-0.035	0.001	#DIV/0!	#DIV/0!	1.000	#DIV/0!
32	0.020	-0.035	-0.034	-0.035	0.001	#DIV/0!	#DIV/0!	1.000	#DIV/0!
33	0.020	-0.035	-0.036	-0.036	0.001	#DIV/0!	#DIV/0!	1.000	#DIV/0!
34	0.020	-0.035	-0.032	-0.034	0.002	#DIV/0!	#DIV/0!	1.000	#DIV/0!
35	0.020	-0.034	-0.027	-0.031	0.005	#DIV/0!	#DIV/0!	1.000	#DIV/0!
36	0.020	-0.034	-0.031	-0.033	0.002	#DIV/0!	#DIV/0!	1.000	#DIV/0!
37	0.020	-0.034	-0.033	-0.034	0.001	#DIV/0!	#DIV/0!	1.000	#DIV/0!
38	0.020	-0.034	-0.034	-0.034	0.000	#DIV/0!	#DIV/0!	1.000	#DIV/0!
39	0.020	-0.032	-0.025	-0.029	0.005	#DIV/0!	#DIV/0!	1.000	#DIV/0!
40	0.020	-0.034	-0.022	-0.028	0.006	#DIV/0!	#DIV/0!	1.000	#DIV/0!



LDL 147 \bar{x} = 5.11
 LDL 148 \bar{x} = 4.66
 oxLDL \bar{x} = 0.198
 (omit 3rd point)
 probably wrong vol.
 pipetted
 IgM below
 detection
 limit

File 1
 Sample
 Solver
 Conc 1
 Dil. 1

SAMPLE
 1
 2

Author's Signature <i>Denise Krul</i>	Date 10/26/96	Read and Understood By <i>Denise Nachowiak</i>	Date 10-1-97
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DATE:
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NUMBER:

Project Number	Subject	Book Number
SEARLE	CETP associated IgM: Binding to α 1OL	GDS - 5734
		Page 057

- No IgM was recovered after the attempt to precipitate it with ammonium sulfate. Since we don't know what's in the Pierce eluting buffer this may have affected precipitation or IgM may have been too dilute for forming good precipitates.
- Will try again.

Another CETP prep from Heidi (8/7/96, see her notebook 6821052) was used to concentrate IgM.

I pooled eluted fractions 4,5,6

Used Centricon-30 to concentrate IgM. Washed the Centricon-30 once w H₂O to remove glycerol. Then concentrated IgM as per manufacturer's instructions. De-salted versus PBS.

----> Quantitation Results Report <----

Date : 10-22-1996
Time : 01:12:40
Operator : Not Entered

File Name : Data not stored yet

Sample Name : Analytical Wavelength : 280 nm
Solvent Name : Reference Wavelength : None Selected
Conc Units : Confirmation Wavelengths : None Selected
Dil. Factor : 1.00 Integration Time : 1 seconds

SAMPLE #	Sample Name	Wavelength	Func. Res.	Concentration
1	blank PBS	Analytical	+0.0000	
2	IgM NRAT (prep from 8/7/96) Heidi Hope Notebook 6821052	Analytical	+0.0583	→ 41.6 μ g/ml

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Book Number GDS - 5734	Subject CETP Associated IgM: Binding to oxLDL	Project Number SEARLE
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Tested ability of the IgM purified from that which was associated with human plasma CETP. and a control human IgM from Sigma (1-8260, lot 085H-4842).

Essentially performed ELISA assays as described by Horkko, S et al. (1996) J. Clin. Invest. 98 (3):815-25.

Used Cardiolipin (C-1649, lot 85H8380 Sigma) to coat one plate. This is because antibodies (Igms) to oxLDL in humans tend to react with the oxidized phospholipids — are seen when you let Cardiolipin coat plastic wells.

- 40 µg/ml cardiolipin stock in 100% EtOH (10mg QS to 250 ml 100% EtOH)
- oxLDL and LDL were used (oxLDL₁₄₇ & LDL₁₄₇) (198 µg/ml) (510 µg/ml) coated plates to 5 µg/ml

Sigma IgM 940 µg/ml 580 µl diluted to 2.41 ml
⇒ 10 µg/ml

CETP IgM 41.6 µg/ml 26 µl QS to 2.40 ml
⇒ 10 µg/ml

Dilutions of IgMs made in 1% BSA, TBS buffer
1.2 ml + 1.2 ml, etc.

Dilute
of
IgM
↑

Author's Signature Elaine Kuhl	Date 10/26/96	Read and Understood By Denise Nachowiak	Date 10-1-97
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-1-97

Project Number	Subject	Book Number
SEARLE	CETP Associated IgM: Binding to oxLDL	GDS - 5734
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Dilutions
of
IgM
↑

DATE: 10/23/96 Plate ①

ASSAY:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0		0									
B	0.156		0.156									
C	0.3125		0.3125									
D	0.625		0.625									
E	1.25		1.25									
F	2.5		2.5									
G	5.0		5.0									
H	10.0		10.0									

hIgM CETPIgM hIgM CETPIgM hIgM CETPIgM
No coat LDL coat oxLDL coat

DATE: Plate ②

ASSAY:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0											
B	0.156											
C	0.3125											
D	0.625											
E	1.25											
F	2.5											
G	5.0											
H	10.0											

hIgM CETPIgM hIgM CETPIgM
Ethanol coat cardiolipin coat

Used
Sigma
A-3437
a human
IgM
APC
conjugate
(μ chain
specific)
Lot
0144-
8904
1:3700x
dilute

Author's Signature	Date	Read and Understood By	Date
Elaine Kuhl	10/26/96	Denise Nachowick	10-1-97

Book Number GDS - 5734	Subject CETP Associated IgM: Binding to oxLDL	Project Number SEARLE
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	1	2	3	4	5	6	7	8	9	10	11	12
A	0.101	0.103	0.100	0.098	0.113	0.101	0.097	0.102	0.093	0.107	0.116	0.112
B	0.121	0.134	0.131	0.107	0.125	0.125	0.109	0.111	0.111	0.127	0.100	0.107
C	0.141	0.115	0.105	0.108	0.143	0.139	0.109	0.105	0.130	0.129	0.106	0.122
D	0.133	0.126	0.100	0.098	0.177	0.171	0.127	0.123	0.155	0.161	0.120	0.118
E	0.164	0.168	0.132	0.110	0.272	0.270	0.166	0.162	0.222	0.213	0.114	0.126
F	0.274	0.248	0.131	0.148	0.413	0.429	0.208	0.247	0.415	0.360	0.145	0.148
G	0.330	0.313	0.150	0.224	0.633	0.645	0.358	0.354	0.598	0.558	0.183	0.272
H	0.558	0.497	0.215	0.290	1.032	0.953	0.764	0.729	0.910	0.923	0.274	0.277

READ DATE:
10/25/96
ASSAY NAME:
PLATE NUMBER:
102596p1
READER NUMBER:

Human IgM

[IgM]	LDL		Mean	No Coat		Mean	Net LDL
0	0.113	0.101	0.107	0.101	0.103	0.102	0.005
0.156	0.125	0.125	0.125	0.121	0.134	0.128	-0.003
0.3125	0.143	0.139	0.141	0.141	0.115	0.128	0.013
0.625	0.177	0.171	0.174	0.133	0.126	0.130	0.045
1.25	0.272	0.270	0.271	0.164	0.168	0.166	0.105
2.5	0.413	0.429	0.421	0.274	0.248	0.260	0.161
5	0.633	0.645	0.639	0.330	0.313	0.322	0.318
10	1.032	0.953	0.993	0.558	0.497	0.528	0.465
						Net	
	oxLDL		Mean	No Coat		Mean	oxLDL
0	0.093	0.107	0.100	0.101	0.103	0.102	-0.002
0.156	0.111	0.127	0.119	0.121	0.134	0.128	-0.009
0.3125	0.130	0.129	0.130	0.141	0.115	0.128	0.002
0.625	0.155	0.161	0.158	0.133	0.126	0.130	0.029
1.25	0.222	0.213	0.218	0.164	0.168	0.166	0.052
2.5	0.415	0.360	0.388	0.274	0.248	0.260	0.128
5	0.598	0.558	0.578	0.330	0.313	0.322	0.257
10	0.910	0.923	0.917	0.558	0.497	0.528	0.389

CETP IgM

[IgM]	LDL		Mean	No Coat		Mean	Net LDL
0	0.097	0.102	0.100	0.100	0.096	0.098	0.002
0.156	0.109	0.111	0.110	0.131	0.107	0.119	-0.009
0.3125	0.109	0.105	0.107	0.105	0.106	0.106	0.002
0.625	0.127	0.123	0.125	0.100	0.096	0.098	0.027
1.25	0.188	0.162	0.174	0.132	0.110	0.121	0.053
2.5	0.208	0.247	0.228	0.131	0.148	0.140	0.088
5	0.358	0.354	0.356	0.150	0.224	0.187	0.169
10	0.764	0.729	0.747	0.215	0.290	0.253	0.494
						Net	
	oxLDL		Mean	No Coat		Mean	oxLDL
0	0.116	0.112	0.114	0.100	0.096	0.098	0.016
0.156	0.100	0.107	0.104	0.131	0.107	0.119	-0.016
0.3125	0.108	0.122	0.114	0.105	0.106	0.106	0.008
0.625	0.120	0.118	0.119	0.100	0.096	0.098	0.021
1.25	0.114	0.126	0.120	0.132	0.110	0.121	-0.001
2.5	0.145	0.148	0.147	0.131	0.148	0.140	0.007
5	0.183	0.272	0.228	0.150	0.224	0.187	0.041
10	0.274	0.277	0.276	0.215	0.290	0.253	0.023

Author's Signature <i>Elaine Kuhl</i>	Date <i>10/26/96</i>	Read and Understood By <i>Dennis Nachowiak</i>	Date <i>10-1-97</i>
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Project Number	Subject	Book Number
SEARLE	CETP Associated IgM: Binding to oxLDL	GDS - 5734
		Page 061

ATE:
98
AME:
MBER:
ip1
JMBER:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.101	0.103	0.104	0.093	0.092	0.091	0.090	0.093	0.010	0.009	0.010	0.010
B	0.106	0.101	0.098	0.094	0.094	0.100	0.091	0.088	0.011	0.009	0.010	0.008
C	0.111	0.109	0.104	0.104	0.109	0.113	0.098	0.091	0.013	0.009	0.012	0.012
D	0.129	0.121	0.108	0.105	0.111	0.118	0.098	0.103	0.019	0.008	0.011	0.010
E	0.144	0.127	0.119	0.112	0.121	0.117	0.097	0.098	0.010	0.009	0.012	0.012
F	0.212	0.191	0.138	0.128	0.138	0.160	0.103	0.115	0.008	0.011	0.009	0.008
G	0.252	0.184	0.148	0.171	0.178	0.185	0.162	0.173	0.013	0.009	0.009	0.008
H	0.461	0.408	0.241	0.224	0.247	0.239	0.188	0.219	0.009	0.008	0.004	0.007

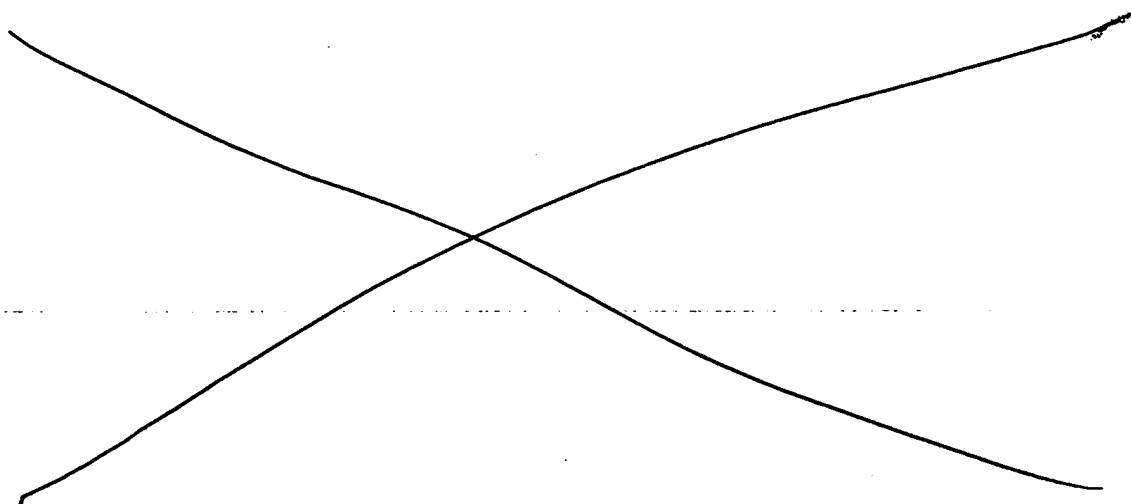
READ DATE:
10/25/96
ASSAY NAME:
PLATE NUMBER:
102596p2
READER NUMBER:

Human IgM

[IgM]	Cardiolipin		Mean	EtOH coat		Mean	Net Cardiolipin
0	0.092	0.091	0.092	0.101	0.103	0.102	-0.011
0.156	0.094	0.100	0.097	0.106	0.101	0.104	-0.007
0.3125	0.109	0.113	0.111	0.111	0.109	0.110	0.001
0.625	0.111	0.118	0.115	0.129	0.121	0.125	-0.011
1.25	0.121	0.117	0.119	0.144	0.127	0.136	-0.017
2.5	0.138	0.160	0.149	0.212	0.191	0.202	-0.053
5	0.178	0.185	0.182	0.252	0.194	0.223	-0.042
10	0.247	0.239	0.243	0.461	0.408	0.435	-0.192

CETP IgM

[IgM]	Cardiolipin		Mean	EtOH coat		Mean	Net Cardiolipin
0	0.090	0.093	0.092	0.104	0.093	0.099	-0.007
0.156	0.091	0.088	0.090	0.096	0.094	0.095	-0.006
0.3125	0.096	0.091	0.094	0.104	0.104	0.104	-0.011
0.625	0.098	0.103	0.101	0.106	0.105	0.106	-0.005
1.25	0.097	0.098	0.098	0.119	0.112	0.116	-0.018
2.5	0.103	0.115	0.109	0.136	0.128	0.132	-0.023
5	0.162	0.173	0.168	0.148	0.171	0.160	0.008
10	0.186	0.219	0.203	0.241	0.224	0.233	-0.030

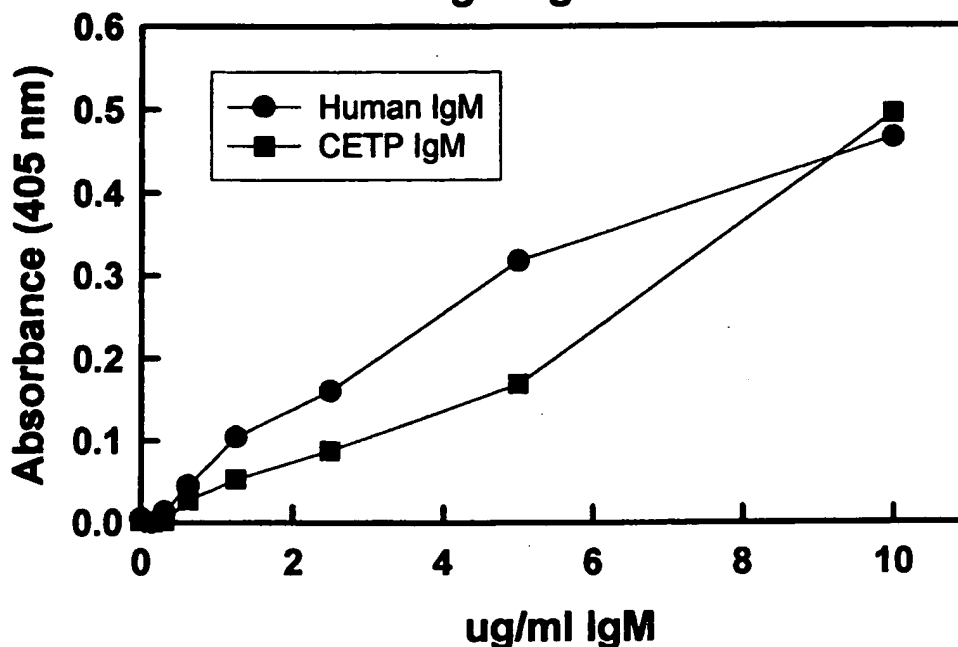


-1-97

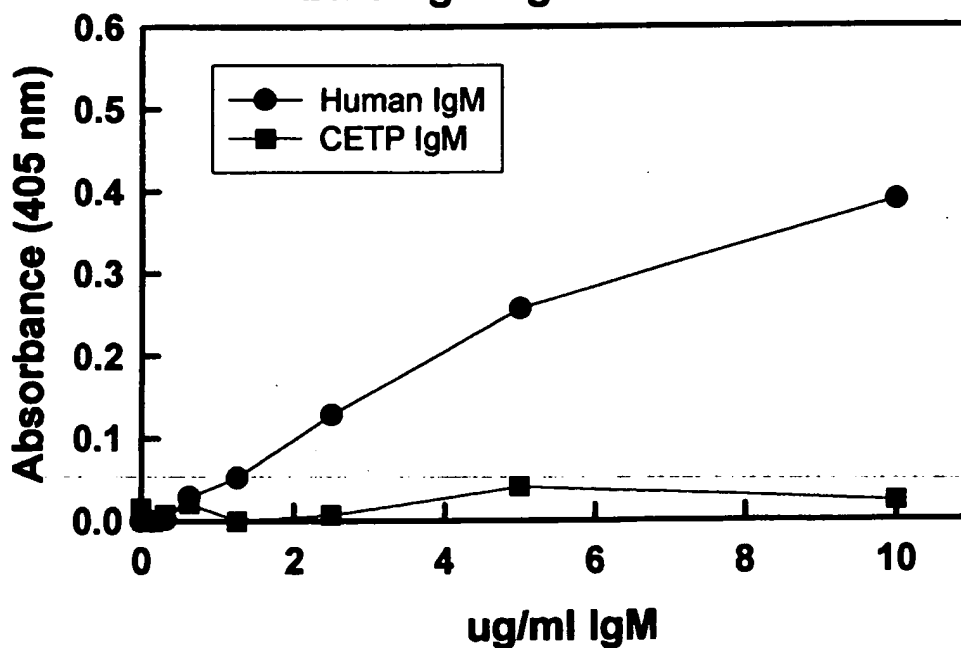
Author's Signature	Date	Read and Understood By	Date
Elaine Kuhl	10/26/96	Denise Nachowiak	10-1-97

Book Number GDS - 5734	Subject <i>CETP Associated IgM: Binding to oxLDL</i>	Project Number SEARLE
Page 062		

Binding of IgMs to LDL



Binding of IgMs to oxLDL



Author's Signature <i>Elaine Kuhl</i>	Date <i>10/26/96</i>	Read and Understood By <i>Denise Nachoruk</i>	Date <i>10-1-97</i>
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Project Number	Subject	Book Number
SEARLE	CETP Associated IgM: Binding to oxLDL	GDS - 5734
		Page 063

Conclusions:

- ① The LDL₁₇ probably has some modifications/oxidative epitopes on it such that a commercial source of human IgM has the ability to bind to it. I didn't use an irrelevant immunoglobulin (IgM directed to a specific antigen) as a control. Next time should do this. Problem is that most human IgMs should bind to oxLDL — use very fresh LDL next time too as a control.
 - ② The CETP assoc. IgM bound almost equally on a $\mu\text{g/ml}$ basis to the LDL — therefore does not have a proportion of Abs to a specific LDL epitope, but appears to have some affinity for LDL.
 - ③ The Human IgM bound virtually equally to oxLDL as LDL untreated. Therefore the epitopes recognized were sufficient on the starting LDL & CuSO₄ oxidation did not enhance or lose any epitopes.
 - ④ CETP IgM, on the other hand, ~~did~~ did not bind oxLDL suggesting that Cu²⁺ oxidation destroyed the epitope recognized (or it was eliminated by the dialysis step or dilution step, etc.)
- ∴ The CETP IgM appears to recognize something specific on LDL. Is it lipid or protein? Oxidized lipid? Or lipid-protein complex?

Author's Signature	Date	Read and Understood By	Date
Elaine Kuhl	10/26/96	Denise Nachowiak	10-1-97

Book Number GDS - 5734	Subject Lipid Assays on Anthrax from CETP Peptide Immunized Rabbit	Project Number SEARLE
Page 064		

Project Number S

1 Rab01 pre 10/27/95			
2 Rab01 12/4/95			
3 Rab01 3/8/96			
4 Rab02 pre 10/27/95			
5 Rab02 12/4/95			
6 Rab02 3/8/96			
7 Rab02 10/25/96			
8 Rab03 pre 10/27/95			
9 Rab03 12/4/95			
10 Rab04 pre 10/27/95			
11 Rab04 12/4/95			
12 Rab05 pre 10/27/95			
13 Rab05 12/4/95			
14 Rab06 pre 10/27/95			
15 Rab06 12/4/95			
16 Rab07 pre 10/27/95			
17 Rab07 12/4/95			
18 Rab07 3/8/96			
19 Rab07 10/25/96			
20 Rab08 pre 10/27/95			
21 Rab08 12/4/95			
22 Rab08 3/8/96			
23 Rab08 10/25/96			
24 Rab09 pre 10/27/95			
25 Rab09 12/4/95			
26 Rab10 pre 10/27/95			
27 Rab10 12/4/95			
28 Rab1 pre 5/30/95			
29 Rab1 7/5/95			
30 Rab1 10/5/95			
31 Rab1 3/8/96			
32 Rab2 pre 5/30/95			
33 Rab2 7/5/95			
34 Rab2 10/5/95			
35 Rab2 3/8/96			
36 Rab3 pre 5/30/95			
37 Rab3 7/5/95			
38 Rab3 8/9/95			
39 Rab4 pre 5/30/95			
40 Rab4 7/5/95			
41 Rab4 8/9/95			
42 Rab5 pre 5/30/95			
43 Rab5 7/5/95			
44 Rab5 8/9/95			
45 Rab6 pre 5/30/95			
46 Rab6 7/5/95			
47 Rab6 8/9/95			
48 Rab7 pre 5/30/95			
49 Rab7 7/5/95			
50 Rab7 8/9/95			
51 Rab8 pre 5/30/95			
52 Rab8 7/5/95			
53 Rab8 8/9/95			
54 Rab9 pre 5/30/95			
55 Rab9 7/5/95			
56 Rab9 8/9/95			
57 Rab10 pre 5/30/95			
58 Rab10 7/5/95			
59 Rab10 8/9/95			
60 Rab11 pre 5/30/95			
61 Rab11 7/5/95			
62 Rab11 10/5/95			
63 Rab11 3/8/96			
64 Rab12 pre 5/30/95			
65 Rab12 7/5/95			
66 Rab12 10/5/95			
67 Rab12 3/8/96			
68			

10/27/95 → 12/4/95 38 days
 12/4/95 → 3/8/96 95 → 133 days
 3/8/96 → 10/25/96 231 → 364 days

5/30/95 → 7/5/95 36 days
 7/5/95 → 10/5/95 92 → 128 days
 10/5/95 → 3/8/96 155 → 283 days
 → 7/5/95 → 8/9/95 35 → 71 days

Ag carrier MAP Pre-Bloods 5/30/95 0
CETP CFA 6/1/95 2
Peptide IFA 6/22/95 23
Immunizations IFA 9/21/95 114
 IFA 2/23/96 269

Ag carrier PPD Pre-Bloods 10/27/95 8
CETP CFA 11/1/95 5
Peptide IFA 11/22/95 26
Immunizations IFA 2/23/96 119
 IFA 10/12/96 351

Author's Signature Elaine Kuhl	Date 12/2/96	Read and Understood By Denise Nachowiak	Date 10-1-97
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Author's Signature E

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days
33 days
64 day

15 day
3 day

day

75 0
5 2
75 23
75 114
76 269

278 8
5 5
75 26
76 119
76 351

Project Number	Subject	Book Number
SEARLE	Lipid Assay on Anthrax from C57P-Pepide Immunized Rabbit	GDS - 5734
		Page 065

60ul sample Tot Chol & HDL Plate ①

DATE:	11/14/96											
ASSAY:												
	1	2	3	4	5	6	7	8	9	10	11	12
A	5700		1		9		17		25		BLK	
B			2		10		18		26		BLK	
C			3		11		19		27		CH	
D			4		12		20		28		C2	
E			5		13		21		29		X	
F			6		14		22		30		X	
G			7		15		23		31		X	
H			8		16		24		32		X	

Tot Chol & HDL Plate ②

DATE:	11/14/96											
ASSAY:												
	1	2	3	4	5	6	7	8	9	10	11	12
A	5700		33		41		49		57		65	
B			34		42		50		58		66	
C			35		43		51		59		67	
D			36		44		52		60		BLK	
E			37		45		53		61		BLK	
F			38		46		54		62		C1	
G			39		47		55		63		C2	
H			40		48		56		64		BLK	

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Author's Signature	Date	Read and Understood By	Date
Elaine Kuhl	12/2/96	Denise Nachowiak	10-1-97

Project

READ DATE:	11/14/96
ASSAY NAME:	111496p1
PLATE NUMBER:	
READER NUMBER:	

SAMPLE RESULTS (DUPLICATES)

STD CURVE

$$y = 0.0728x + 0.006$$

$$R^2 = 0.9994$$

ug CHOL (x)	ABS 500nm (y)
0	0.006
1	0.150
2	0.294
3	0.438
4	0.582
5	0.726
6	0.870

(ACTUAL)
191
381

Author

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RLE

DATE:

11/4/96

Y NAME:

496p1

NUMBER:

NUMBER:

Project Number	Subject	Book Number
SEARLE	Lipid Assay on Antisera... continued	GDS - 5734
		Page 067

	1	2	3	4	5	6	7	8	9	10	11	12
A	-0.001	0.001	0.194	0.182	0.101	0.108	0.162	0.158	0.175	0.174	0.091	0.084
B	0.041	0.049	0.091	0.092	0.156	0.160	0.124	0.131	0.107	0.107	0.089	0.075
C	0.087	0.084	0.047	0.048	0.095	0.101	0.147	0.145	0.143	0.133	0.060	0.059
D	0.160	0.174	0.130	0.131	0.058	0.060	0.100	0.100	0.207	0.212	0.005	0.005
E	0.319	0.318	0.078	0.079	0.136	0.140	0.082	0.079	0.114	0.118	0.003	0.005
F	0.452	0.460	0.078	0.080	0.105	0.104	0.321	0.323	0.078	0.075	0.213	0.211
G	0.584	0.602	0.178	0.168	0.080	0.082	0.191	0.194	0.074	0.073	0.391	0.373
H	0.733	0.753	0.100	0.100	0.231	0.249	0.171	0.167	0.137	0.139	0.003	0.005

READ DATE:

11/14/96

ASSAY NAME:

111496p2

PLATE NUMBER

READER NUMBER:

CHOLESTEROL ASSAY

ug	OD 1	OD 2	MEAN	SD								CALC
STD	OD	OD	OD	OD	m	b						STD
0	-0.001	0.001	0.000	0.001								-0.174
0.5	0.041	0.049	0.045	0.008	0.0733	0.0127	#N/A	#N/A	#N/A	#N/A	#N/A	0.440
1	0.087	0.094	0.091	0.005	0.0009	0.0048	#N/A	#N/A	#N/A	#N/A	#N/A	1.060
2	0.160	0.174	0.167	0.010	0.9991	0.0087	#N/A	#N/A	#N/A	#N/A	#N/A	2.104
4	0.319	0.318	0.318	0.002	#####	8.000	#N/A	#N/A	#N/A	#N/A	#N/A	4.158
8	0.452	0.460	0.456	0.008	0.523	0.000	#N/A	#N/A	#N/A	#N/A	#N/A	8.044
8	0.584	0.602	0.593	0.013								7.912
10	0.733	0.753	0.743	0.014								9.958

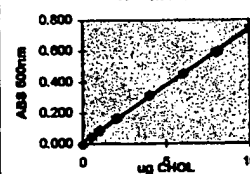
SAMPLE RESULTS (DUPLICATES)

SAMP.	NO.	(ml)	OD 1	OD 2	MEAN	SD	CALC.	CALC.	DF	mg/dl
			OD	OD	OD	OD	ug	ug/ml		CHOL
	33	0.060	0.194	0.182	0.188	0.008	2.390	39.831	20,000	79.882
	34	0.060	0.091	0.092	0.092	0.001	1.074	17.900	20,000	35.800
	35	0.060	0.047	0.048	0.048	0.001	0.474	7.901	20,000	15.802
	36	0.060	0.130	0.131	0.131	0.001	1.608	26.763	20,000	53.527
	37	0.060	0.078	0.079	0.079	0.001	0.897	14.948	20,000	29.892
	38	0.060	0.078	0.080	0.079	0.001	0.904	15.059	20,000	30.119
	39	0.060	0.178	0.168	0.171	0.007	2.158	35.967	20,000	71.935
	40	0.060	0.100	0.100	0.100	0.000	1.180	19.832	20,000	39.684
	41	0.060	0.101	0.108	0.104	0.004	1.238	20.627	20,000	41.255
	42	0.060	0.156	0.160	0.158	0.003	1.981	33.013	20,000	68.028
	43	0.060	0.085	0.101	0.098	0.004	1.163	19.377	20,000	38.755
	44	0.060	0.056	0.060	0.058	0.003	0.817	10.287	20,000	20.574
	45	0.060	0.136	0.140	0.138	0.003	1.708	28.468	20,000	56.936
	46	0.060	0.105	0.104	0.105	0.001	1.251	20.855	20,000	41.709
	47	0.060	0.080	0.082	0.081	0.001	0.931	15.514	20,000	31.028
	48	0.060	0.231	0.249	0.240	0.013	3.099	51.648	20,000	103.297
	49	0.060	0.162	0.159	0.161	0.002	2.015	33.581	20,000	67.162
	50	0.060	0.124	0.131	0.128	0.005	1.565	28.082	20,000	52.163
	51	0.060	0.147	0.145	0.146	0.001	1.817	30.288	20,000	60.572
	52	0.060	0.100	0.100	0.100	0.000	1.190	19.832	20,000	39.684
	53	0.060	0.082	0.079	0.081	0.002	0.924	15.400	20,000	30.801
	54	0.060	0.321	0.323	0.322	0.001	4.217	70.284	20,000	140.568
	55	0.060	0.191	0.194	0.193	0.002	2.451	40.854	20,000	81.707
	56	0.060	0.171	0.167	0.169	0.003	2.131	35.513	20,000	71.026
	57	0.060	0.175	0.174	0.175	0.001	2.208	36.763	20,000	73.528
	58	0.060	0.107	0.107	0.107	0.000	1.285	21.423	20,000	42.846
	59	0.060	0.143	0.133	0.138	0.007	1.708	28.468	20,000	56.936
	60	0.060	0.207	0.212	0.210	0.004	2.683	44.717	20,000	89.434
	61	0.060	0.114	0.116	0.115	0.001	1.394	23.241	20,000	48.482
	62	0.060	0.076	0.075	0.076	0.001	0.856	14.284	20,000	28.528
	63	0.060	0.074	0.073	0.074	0.001	0.829	13.810	20,000	27.619
	64	0.060	0.137	0.136	0.138	0.001	1.708	28.468	20,000	56.936
	65	0.060	0.091	0.094	0.093	0.002	1.088	18.127	20,000	36.255
	66	0.060	0.069	0.075	0.072	0.004	0.808	13.469	20,000	26.937
	67	0.060	0.060	0.069	0.060	0.001	0.638	10.828	20,000	21.256
	blank	0.060	0.005	0.005	0.005	0.000	-0.105	-1.758	20,000	-3.516
	blank	0.060	0.003	0.005	0.004	0.001	-0.119	-1.985	20,000	-3.970
	Cardio1	0.060	0.213	0.211	0.212	0.001	2.717	45.285	40,000	181.141
	Cardio2	0.060	0.391	0.373	0.382	0.013	5.035	83.919	40,000	335.678
	blank	0.060	0.003	0.005	0.004	0.001	-0.119	-1.985	20,000	-3.970

STD CURVE

$$y = 0.0733x + 0.0127$$

$$R^2 = 0.9991$$



(ACTUAL)

191

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Author's Signature

Claire Kuhl

Date

12/2/96

Read and Understood By

Denise Nachowisk

Date

10-1-97

Book Number GDS - 5734	Subject <i>Lipid Assay on Anthrax from CETP-Phosphate Immunized Rabbits</i>	Project Number SEARLE
Page 068		

	1	2	3	4	5	6	7	8	9	10	11	12
A	-0.001	0.002	0.221	0.228	0.138	0.139	0.100	0.108	0.104	0.108	0.002	0.001
B	0.043	0.055	0.085	0.100	0.135	0.134	0.135	0.130	0.213	0.215	0.004	0.003
C	0.084	0.083	0.208	0.207	0.098	0.104	0.120	0.120	0.088	0.089	0.188	0.201
D	0.169	0.178	0.305	0.303	0.155	0.155	0.112	0.115	0.279	0.271	0.390	0.391
E	0.318	0.331	0.237	0.242	0.078	0.083	0.088	0.090	0.177	0.178	0.003	0.005
F	0.446	0.455	0.211	0.211	0.112	0.112	0.074	0.075	0.108	0.108	0.003	0.008
G	0.598	0.605	0.209	0.209	0.087	0.101	0.080	0.081	0.129	0.128	0.004	0.004
H	0.738	0.744	0.208	0.209	0.218	0.220	0.218	0.198	0.219	0.218	0.002	0.004

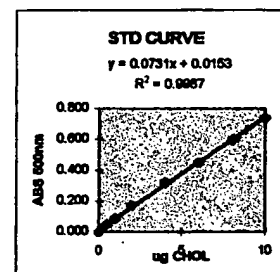
READ DATE:
11/14/98
ASSAY NAME:
111498p3
PLATE NUMBER:
READER NUMBER:

HDL CHOLESTEROL ASSAY

ug STD	OD 1	OD 2	MEAN OD	SD OD								CALC STD
0	-0.001	0.002	0.001	0.002								-0.203
0.5	0.043	0.055	0.049	0.008	m	b						0.480
1	0.084	0.083	0.089	0.008	0.0011	0.0058	#N/A	#N/A	#N/A	#N/A	#N/A	1.001
2	0.169	0.178	0.174	0.008	0.9987	0.0108	#N/A	#N/A	#N/A	#N/A	#N/A	2.163
4	0.318	0.331	0.324	0.011	0.520	0.001	#N/A	#N/A	#N/A	#N/A	#N/A	4.214
8	0.446	0.455	0.451	0.008			#N/A	#N/A	#N/A	#N/A	#N/A	5.851
8	0.598	0.605	0.601	0.008			#N/A	#N/A	#N/A	#N/A	#N/A	8.003
10	0.738	0.744	0.740	0.008			#N/A	#N/A	#N/A	#N/A	#N/A	9.911

SAMPLE RESULTS (DUPLICATES)

SAMP. NO.	(ml)	OD 1	OD 2	MEAN OD	SD OD	CALC. ug	CALC. ug/ml	DF	mg/dl CHOL
1	0.080	0.221	0.228	0.224	0.004	2.847	47.447	5.500	26.098
2	0.080	0.095	0.100	0.098	0.004	1.124	18.727	5.500	10.300
3	0.080	0.208	0.207	0.208	0.001	2.828	43.800	5.500	24.090
4	0.080	0.305	0.303	0.304	0.001	3.948	65.798	5.500	36.188
5	0.080	0.237	0.242	0.240	0.004	3.088	51.084	5.500	28.102
6	0.080	0.211	0.211	0.211	0.000	2.878	44.588	5.500	24.529
7	0.080	0.209	0.209	0.209	0.000	2.849	44.142	5.500	24.278
8	0.080	0.208	0.209	0.208	0.002	2.828	43.800	5.500	24.090
9	0.080	0.138	0.139	0.139	0.001	1.684	28.073	5.500	15.440
10	0.080	0.135	0.134	0.135	0.001	1.830	27.181	5.500	14.938
11	0.080	0.098	0.104	0.101	0.004	1.171	19.525	5.500	10.739
12	0.080	0.155	0.155	0.155	0.000	1.910	31.833	5.500	17.508
13	0.080	0.078	0.083	0.080	0.005	0.877	14.824	5.500	8.043
14	0.080	0.112	0.112	0.112	0.000	1.322	22.032	5.500	12.118
15	0.080	0.097	0.101	0.099	0.003	1.144	19.069	5.500	10.488
16	0.080	0.218	0.220	0.218	0.003	2.772	46.193	5.500	25.408
17	0.080	0.100	0.108	0.103	0.004	1.189	19.881	5.500	10.889
18	0.080	0.135	0.130	0.133	0.004	1.602	26.705	5.500	14.688
19	0.080	0.120	0.120	0.120	0.000	1.431	23.856	5.500	13.121
20	0.080	0.112	0.115	0.114	0.002	1.342	22.374	5.500	12.308
21	0.080	0.088	0.090	0.088	0.003	0.994	16.562	5.500	9.109
22	0.080	0.074	0.075	0.075	0.001	0.809	13.485	5.500	7.417
23	0.080	0.080	0.081	0.081	0.001	0.818	10.284	5.500	5.881
24	0.080	0.216	0.198	0.207	0.013	2.821	43.688	5.500	24.027
25	0.080	0.104	0.108	0.108	0.003	1.240	20.665	5.500	11.368
26	0.080	0.213	0.215	0.214	0.001	2.717	45.282	5.500	24.805
27	0.080	0.088	0.089	0.089	0.001	1.001	16.878	5.500	9.172
28	0.080	0.279	0.271	0.275	0.008	3.551	59.188	5.500	32.552
29	0.080	0.177	0.178	0.178	0.001	2.218	38.982	5.500	20.329
30	0.080	0.108	0.108	0.108	0.000	1.267	21.120	5.500	11.818
31	0.080	0.129	0.128	0.129	0.001	1.548	25.793	5.500	14.188
32	0.080	0.219	0.218	0.219	0.001	2.778	46.307	5.500	25.489
blank	0.080	0.002	0.001	0.002	0.001	-0.189	-3.155	5.500	-1.735
blank	0.080	0.004	0.003	0.004	0.001	-0.182	-2.689	5.500	-1.484
Cardio1	0.080	0.188	0.201	0.195	0.009	2.450	40.837	40.000	163.348
Cardio2	0.080	0.390	0.391	0.391	0.001	5.131	85.512	40.000	342.050
blank	0.080	0.003	0.005	0.004	0.001	-0.155	-2.585	20.000	-5.170
blank	0.080	0.003	0.008	0.005	0.002	-0.148	-2.471	20.000	-4.942
blank	0.080	0.004	0.004	0.004	0.000	-0.155	-2.585	20.000	-5.170
blank	0.080	0.002	0.004	0.003	0.001	-0.169	-2.813	20.000	-5.626



(ACTUAL)
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Author's Signature <i>Elaine Krul</i>	Date <i>12/2/96</i>	Read and Understood By <i>Denise Nachowiak</i>	Date <i>10-1-97</i>
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ARLE

READ DATE:
11/14/96
ASSAY NAME:
111496p3
ATE NUMBER
READER NUMBER

Project Number	Subject	Book Number
SEARLE	Lipid Assays on Anthraeia.... Continued	GDS -
		Page 069

	1	2	3	4	5	6	7	8	9	10	11	12
A	-0.002	0.002	0.203	0.204	0.285	0.279	0.182	0.191	0.277	0.278	0.178	0.173
B	0.044	0.050	0.105	0.108	0.248	0.250	0.167	0.168	0.198	0.201	0.165	0.162
C	0.069	0.095	0.083	0.083	0.180	0.173	0.252	0.263	0.288	0.283	0.114	0.115
D	0.157	0.173	0.161	0.160	0.133	0.133	0.157	0.163	0.244	0.244	0.003	0.003
E	0.305	0.320	0.128	0.129	0.268	0.270	0.164	0.169	0.165	0.167	0.008	0.003
F	0.449	0.484	0.165	0.165	0.174	0.174	0.161	0.167	0.149	0.150	0.200	0.199
G	0.589	0.585	0.233	0.232	0.168	0.171	0.150	0.149	0.135	0.138	0.390	0.383
H	0.727	0.734	0.161	0.164	0.183	0.191	0.232	0.223	0.179	0.182	0.005	0.008

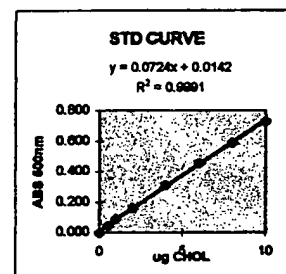
READ DATE:
11/14/96
ASSAY NAME:
111496p4
PLATE NUMBER
READER NUMBER

HDL CHOLESTEROL ASSAY

ug	STD	OD 1	OD 2	MEAN	SD	OD	OD	OD	OD	OD	OD	OD	CALC	STD
0	-0.002	0.002	0.000	0.003		m	b	#N/A	#N/A	#N/A	#N/A	#N/A	-0.198	
0.5	0.044	0.050	0.047	0.004	0.0724	0.0142	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	0.453	
1	0.069	0.095	0.082	0.004	0.0009	0.0048	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	1.074	
2	0.157	0.173	0.165	0.011	0.9991	0.0089	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	2.082	
4	0.305	0.320	0.313	0.011	#####	6.000	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	4.118	
6	0.449	0.484	0.457	0.011	0.510	0.000	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	6.105	
8	0.589	0.585	0.592	0.004									7.978	
10	0.727	0.734	0.731	0.005									9.888	

SAMPLE RESULTS (DUPLICATES)

SAMP.	NO.	(ml)	OD 1	OD 2	MEAN	SD	OD	OD	CALC.	CALC.	DF	mg/dl
	33	0.060	0.203	0.204	0.204	0.001	2.813	43.554	5.500	23.955		
	34	0.060	0.105	0.108	0.107	0.002	1.274	21.239	5.500	11.682		
	35	0.060	0.083	0.083	0.083	0.000	0.850	15.833	5.500	8.708		
	36	0.060	0.161	0.160	0.161	0.001	2.020	33.882	5.500	18.514		
	37	0.060	0.128	0.129	0.128	0.002	1.584	28.070	5.500	14.339		
	38	0.060	0.165	0.165	0.165	0.000	2.082	34.697	5.500	19.083		
	39	0.060	0.233	0.232	0.233	0.001	3.014	50.226	5.500	27.624		
	40	0.060	0.161	0.164	0.163	0.002	2.047	34.122	5.500	18.787		
	41	0.060	0.285	0.279	0.282	0.004	3.697	61.613	5.500	33.887		
	42	0.060	0.248	0.250	0.248	0.001	3.241	54.022	5.500	29.712		
	43	0.060	0.180	0.173	0.177	0.005	2.241	37.343	5.500	20.539		
	44	0.060	0.133	0.133	0.133	0.000	1.640	27.338	5.500	15.035		
	45	0.060	0.268	0.270	0.268	0.003	3.504	58.383	5.500	32.118		
	46	0.060	0.174	0.174	0.174	0.000	2.208	36.768	5.500	20.222		
	47	0.060	0.168	0.171	0.170	0.002	2.144	35.733	5.500	19.653		
	48	0.060	0.193	0.191	0.192	0.001	2.455	40.909	5.500	22.500		
	49	0.060	0.182	0.191	0.187	0.006	2.379	39.843	5.500	21.804		
	50	0.060	0.187	0.188	0.188	0.001	2.382	39.873	5.500	21.930		
	51	0.060	0.252	0.263	0.258	0.008	3.359	55.977	5.500	30.787		
	52	0.060	0.157	0.163	0.160	0.004	2.013	33.547	5.500	18.451		
	53	0.060	0.164	0.169	0.167	0.004	2.103	35.042	5.500	19.273		
	54	0.060	0.161	0.167	0.164	0.004	2.068	34.467	5.500	18.957		
	55	0.060	0.150	0.149	0.150	0.001	1.868	31.131	5.500	17.122		
	56	0.060	0.232	0.223	0.228	0.006	2.945	49.078	5.500	26.992		
	57	0.060	0.277	0.278	0.278	0.001	3.635	60.578	5.500	33.318		
	58	0.060	0.198	0.201	0.200	0.002	2.558	42.834	5.500	23.449		
	59	0.060	0.289	0.293	0.291	0.003	3.821	63.684	5.500	35.026		
	60	0.060	0.244	0.244	0.244	0.000	3.172	52.871	5.500	29.079		
	61	0.060	0.165	0.167	0.166	0.001	2.086	34.927	5.500	19.210		
	62	0.060	0.149	0.150	0.150	0.001	1.868	31.131	5.500	17.122		
	63	0.060	0.135	0.138	0.137	0.002	1.688	28.141	5.500	15.477		
	64	0.060	0.179	0.182	0.181	0.002	2.296	38.263	5.500	21.045		
	65	0.060	0.178	0.173	0.178	0.004	2.227	37.113	5.500	20.412		
	66	0.060	0.165	0.162	0.164	0.002	2.061	34.352	5.500	18.894		
	67	0.060	0.114	0.115	0.115	0.001	1.385	23.080	5.500	12.694		
	blank	0.060	0.003	0.003	0.003	0.000	-0.154	-2.571	5.500	-1.414		
	blank	0.060	0.008	0.003	0.006	0.004	-0.120	-1.998	5.500	-1.086		
	Cardio1	0.060	0.200	0.199	0.200	0.001	2.558	42.834	40.000	170.538		
	Cardio2	0.060	0.390	0.383	0.387	0.005	5.139	85.654	40.000	342.616		
	blank	0.060	0.005	0.008	0.007	0.002	-0.106	-1.786	20.000	-3.532		



(ACTUAL)

191

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Author's Signature	Date	Read and Understood By	Date
<i>Debra Nachowiak</i>	12/2/96	<i>Debra Nachowiak</i>	10-1-97

0-1-97

RLE

Project Number

SEARLE

Subject

CETP Transfer Activity in Sera
from CETP-peptide Immunized
Rabbits

Book Number

GDS - 5734

Page

073

See Ben Kelec Notebook #5748, p. 151 for the
CETP activity assay raw data. Below are the
Summaries.

Assay used 1:20 dilution of rabbit serum
& conducted for 4 hr incubation.

Rabbit 07 below was the only CETP activity that decreased
concomitantly with an elevation of HDL (see previous page).

	RAB SERA	PLATE 1								
	CETP 618			BLANK	10810.5					
	11/27/86									
			CPM	CPM	CPM	% T	% T	% T	AVE % T	ST DEV
		BLANK	10708.3	11038.6	10653					
		BLANK	10233.1	10087.6	10887.8					
		BLANK	11209.7	10987.6	11034.3					
		BLANK	10578.5	10234.2	11084.2					
		BLANK	10876.2	10825	10053.5					
		BLANK	10047.5	10189.8	10902.1					
		POS	2254.77	2740.44	2171.42	78.75	74.17	79.54	77.49	2.90
		NEG	9629.3	9864.26	9518.62	9.25	7.03	10.29	8.86	1.66
1	Rab01 pre 10/27/85		9012.24	9502.85	8241.26	15.06	10.44	22.33	15.94	5.99
2	Rab01 12/4/85		8259.51	8728.84	8249.08	22.16	17.75	22.26	20.72	2.57
3	Rab01 3/8/86		10666.2	9323.15	9538.1	0.42	12.13	10.13	7.56	6.27
4	Rab02 pre 10/27/85		10114.1	8181.17	8412.6	4.68	22.90	20.71	18.10	9.85
5	Rab02 12/4/85		10136	9739.06	8963.88	4.47	8.21	15.33	9.34	5.52
6	Rab02 3/8/86		9302.78	8212.79	8725.57	12.32	13.17	17.78	14.42	2.93
7	Rab02 10/25/86		8419.03	8191.19	8428.62	20.65	22.80	20.58	21.35	1.26
8	Rab03 pre 10/27/85		8782.16	8906.49	8196.75	17.14	16.06	22.75	18.65	3.59
9	Rab03 12/4/85		8743.68	9119.08	9082.86	17.59	14.08	14.30	15.32	1.98
10	Rab04 pre 10/27/85		8119.24	7743.19	8836.78	23.48	27.02	16.72	22.41	5.24
11	Rab04 12/4/85		8825.17	7544.31	8308.22	15.88	28.90	21.70	22.16	6.52
12	Rab05 pre 10/27/85		8381.97	7789.06	8847.97	21.00	26.59	18.50	22.03	4.14
13	Rab05 12/4/85		9813.49	9203.93	9662.6	7.51	13.28	8.83	9.80	2.99
14	Rab06 pre 10/27/85		9846.44	9315.92	9067.38	7.20	12.20	14.54	11.31	3.75
15	Rab06 12/4/85		9620.38	8990.1	9737.27	9.33	15.27	8.23	10.84	3.79
16	Rab07 pre 10/27/85		9309.37	8929.87	8379.61	12.28	15.84	11.60	13.23	2.28
17	Rab07 12/4/85		8378.32	8160.07	8082.44	21.04	23.09	24.01	22.72	1.52
18	Rab07 3/8/86		7948.96	8771.81	8847.07	25.08	17.33	18.50	20.31	4.18
19	Rab07 10/25/86		10046.1	10291.7	10182.9	5.32	3.00	3.94	4.09	1.16
20	Rab08 pre 10/27/85		9051.83	9461.79	9240.05	14.69	10.83	12.92	12.81	1.93
21	Rab08 12/4/85		9255.08	9036.05	9149.94	12.77	14.84	13.77	13.79	1.03
22	Rab08 3/8/86		8774.37	9684.1	8833.92	17.30	6.85	16.74	13.63	5.88
23	Rab08 10/25/86		9258.79	10007.7	9752.97	12.74	5.68	8.08	8.83	3.59
24	Rab08 pre 10/27/85		9322.28	9849.85	8886.41	12.14	7.17	16.25	11.85	4.55

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to appear
CETP-7 imp.

1-97

Author's Signature

Claire Kuhl

Date

12/2/86

Read and Understood By

Denise Nachowiak

Date

10-1-97

Book Number GDS - 5734	Subject CETP transfer Activity & Antibody from CETP-Pepptide Immunized Rabbit	Project Number SEARLE
Page 074		

RAB SERA	PLATE 2								
CETP618			BLANK	10503.0					
11/27/96									
		CPM	CPM	CPM	% T	% T	% T	AVE % T	ST DEV
	BLANK	11282.2	10339.9	10494.8					
	BLANK	11017.4	11239.6	9986.81					
	BLANK	11006.6	11586.5	10983.2					
	BLANK	11114.8	10649.8	10276.2					
	BLANK	7210.34	10757.7	10340.8					
	BLANK	9808.3	10603.1	10356.1					
	POS	1973.51	2451.96	2474.61	81.21	76.65	76.44	78.10	2.69
	NEG	9809.02	10570.6	9869.69	6.61	-0.64	6.03	4.00	4.03
25	Rab09 12/4/95	9585.36	8612.11	8683.51	8.74	18.00	17.32	14.69	5.16
26	Rab010 pre 10/27/95	9191.15	8489.56	8887.09	12.49	19.17	15.39	15.68	3.35
27	Rab010 12/4/95	8071.4	7608.58	7770.91	23.15	27.56	26.01	25.57	2.24
28	Rab1 pre 5/30/95	8363.19	7508.84	8132.82	20.37	28.51	22.57	23.82	4.21
29	Rab1 7/5/95	9113.94	8269.4	8413.38	13.23	21.27	19.90	18.13	4.30
30	Rab1 10/5/95	8318.46	8156.34	8187.48	20.80	22.34	22.05	21.73	0.82
31	Rab1 3/8/96	10379.7	8945.67	9019.57	1.17	14.83	14.12	10.04	7.69
32	Rab2 pre 5/30/95	8298.45	8121.16	8340.71	20.99	22.68	20.59	21.42	1.11
33	Rab2 7/5/95	7370.99	8480.69	8384.95	29.82	19.25	20.17	23.08	5.85
34	Rab2 10/5/95	7666.33	8402.68	8714.38	27.01	20.00	17.03	21.35	5.12
35	Rab2 3/8/96	9285.39	9895.01	9315.07	11.59	5.79	11.31	9.56	3.27
36	Rab3 pre 5/30/95	9571.67	9752.46	9427.22	8.87	7.15	10.24	8.75	1.55
37	Rab3 7/5/95	9066.08	9559.74	9427.08	13.68	8.98	10.24	10.97	2.43
38	Rab3 8/9/95	8999.02	9667.69	8402.9	14.32	7.95	20.00	14.09	6.02
39	Rab4 pre 5/30/95	8019.45	8212.66	7808.4	23.65	21.81	25.66	23.70	1.93
40	Rab4 7/5/95	9453.04	9090.95	8085.25	10.00	13.44	23.21	15.55	6.85
41	Rab4 8/9/95	8089.22	9194.37	8746.71	22.98	12.46	16.72	17.39	5.29
42	Rab5 pre 5/30/95	8829.54	9717.02	8952.29	15.93	7.48	14.76	12.73	4.58
43	Rab5 7/5/95	8516.51	9987.64	9604.42	18.91	4.91	8.56	10.79	7.27
44	Rab5 8/9/95	9072.69	9874.18	9412.75	13.62	5.99	10.38	10.00	3.83
45	Rab6 pre 5/30/95	8469.02	9238.24	9653.13	19.37	12.04	8.09	13.17	5.72
46	Rab6 7/5/95	8991.33	9504.15	9788.33	14.39	9.51	6.80	10.24	3.85
47	Rab6 8/9/95	7722.64	8505.77	8486.19	26.47	19.02	19.20	21.56	4.25
48	Rab7 pre 5/30/95	8240.6	9020.81	8988.58	21.54	14.11	14.42	16.69	4.20

Rabbit 2 showed ↓ CETP at fast blood. This rabbit had consistent decrease in total cholesterol - indicating either poor health or overall ↓ lipoprotein synthesis & this may account for ↓ CETP activity (low CETP may be due to ↓ serum cholesterol). Other Rabbit #1 did not show this, ∴ probably unrelated to immunization.

Author's Signature Elaine Kuhl	Date 12/2/96	Read and Understood By Denise Nachowiak	Date 10-1-97
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Author's
E

RLE

Project Number	Subject	Book Number
SEARLE	CETP Transfer Activity in Antisera from CETP-Replete Immunized Rabbits	GDS - 5734
		Page 075

RAB SERA	PLATE 3								
CETP#18			BLANK	10641.7					
11/27/96									
		CPM	CPM	CPM	% T	% T	% T	AVE % T	ST DEV
	BLANK	11755	11175.2	11040.6					
	BLANK	10591.1	10163.1	11345.6					
	BLANK	10656.6	10384.9	11051.5					
	BLANK	9925.84	9460.2	10845.5					
	BLANK	10694.9	10054.4	10636.4					
	BLANK	10326.9	10346.4	11097.2					
	POS	2089.87	2422.13	2399.78	80.36	77.24	77.45	78.35	1.75
	NEG	10534.1	9918.48	9540.3	1.01	6.80	10.35	6.05	4.71
49 Rab7 7/5/95		9237.54	8913	8333.44	13.20	16.24	21.69	17.04	4.30
50 Rab7 8/9/95		8258.68	8941.53	8852.33	22.39	15.98	16.82	18.40	3.49
51 Rab8 pre 5/30/95		8512.38	8896.61	8121.48	20.01	16.40	23.68	20.03	3.64
52 Rab8 7/5/95		9413.3	8910.75	9439.19	11.54	16.27	11.30	13.04	2.80
53 Rab8 8/9/95		8694.87	8989.74	8716.37	18.29	15.52	18.09	17.30	1.54
54 Rab9 pre 5/30/95		7720.48	8551.09	7977.01	27.45	19.65	25.04	24.05	4.00
55 Rab9 7/5/95		7750.23	8723.74	9015.18	27.17	18.02	15.28	20.16	6.22
56 Rab9 8/9/95		8055.35	8291.32	7873.01	24.30	22.09	26.02	24.14	1.97
57 Rab10 pre 5/30/95		8579.67	7909.08	8875	19.38	25.68	16.60	20.55	4.65
58 Rab10 7/5/95		9027.55	8162.52	8941.2	15.17	23.30	15.98	18.15	4.48
59 Rab10 8/9/95		8578.41	8976.45	8962.59	19.39	15.65	15.78	16.94	2.12
60 Rab11 pre 5/30/95		8344.44	8058.18	8349.21	21.59	24.28	21.54	22.47	1.57
61 Rab11 7/5/95		9097.25	9635.55	9635.24	14.51	9.46	9.46	11.14	2.92
62 Rab11 10/5/95		9932.16	9223.43	9531.58	6.67	13.33	10.43	10.14	3.34
63 Rab11 3/8/96		10474.1	9505.89	9884.74	1.58	10.67	7.11	6.45	4.58
64 Rab12 pre 5/30/95		9460.02	8476.52	8583.1	11.10	20.35	19.34	16.93	5.07
65 Rab12 7/5/95		8598.62	10379.7	9104.66	19.20	2.46	14.44	12.04	8.62
66 Rab12 10/5/95		7502.73	9091.06	7972.8	29.50	14.57	25.08	23.05	7.67
67 Rab12 3/8/96		8929.18	10351.3	9343.77	16.09	2.73	12.20	10.34	6.87
		10594	11717	11683.6	0.45	-10.10	-9.79	-6.48	6.00
		10874.6	12001.5	11596.5	-2.19	-12.78	-8.97	-7.98	5.36
		9719.78	11477.5	10783.6	8.66	-7.85	-1.33	-0.17	8.32
		10828.9	11947	10791.4	-1.76	-12.27	-1.41	-5.14	6.17
		11358.3	12153.7	11568.4	-6.73	-14.21	-8.71	-9.88	3.87

Rabbit 11 showed ↓ CETP activity.
See p. 071 to note that HDL was not elevated
in this rabbit. Total cholesterol dropped over time.
This may contribute to ↓ CETP mass due to ↓ serum
cholesterol.

Note: Can't really understand why cholesterol
appear to drop over time for most rabbits. May
be diet switch, but seems to take a long time.

Author's Signature	Date	Read and Understood By	Date
Elaine Kuhl	12/2/96	Denise Nachowiak	10-1-97

-1-97

Book Number GDS - 5734	Subject <i>FPLC Analysis on Rabbit 08 from CERP-Pepptide Immunizations</i>	Project Number SEARLE
Page 076		

Performed on 10/31/96.

375 μ l serum added to 375 μ l EDTA-saline

↓
filtered thru 0.22 μ m

500 μ l applied to column (\therefore 250 μ l of serum
applied on column)

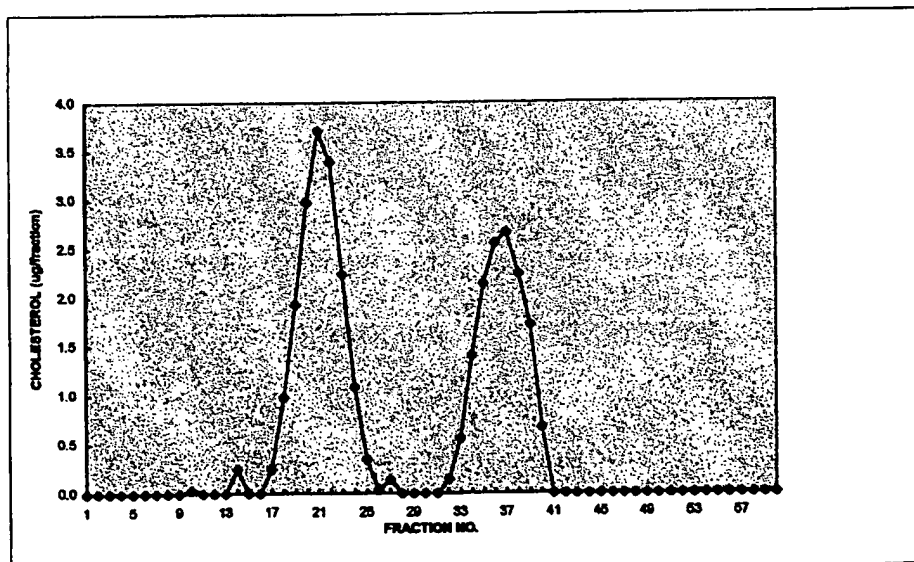
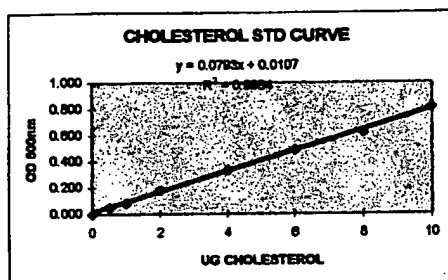
Collected 500 μ l fractions, used 60 μ l for assay.

Author's Signature <i>Clairie Kuhl</i>	Date <i>12/2/96</i>	Read and Understood By <i>Denise Nachowisk</i>	Date <i>10-1-97</i>
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Project Number	Subject <i>EPIC Analysis on Rabbit 08 from CPTP-Pepside. Immunizations</i>	Book Number GDS - 5734
SEARLE		Page 077

	1	2	3	4	5	6	7	8	9	10	11	12	READ DATE:
-0.003	0.003	-0.003	0.008	0.013	0.014	0.016	0.001	0.001	0.001	-0.008	-0.008	-0.010	11/1/86
0.047	0.054	0.003	0.011	0.020	0.011	0.024	0.005	-0.001	-0.003	-0.010	-0.010	-0.010	ASSAY NAME:
0.086	0.091	0.005	0.001	0.029	0.012	0.031	0.005	-0.001	-0.001	-0.010	-0.010	-0.010	
0.174	0.192	-0.001	0.003	0.039	0.005	0.035	0.005	0.001	0.001	-0.008	-0.010	-0.010	PLATE NUMBER:
0.326	0.342	-0.001	0.001	0.046	-0.003	0.036	-0.001	-0.001	-0.001	-0.008	-0.010	-0.010	110196p1
0.473	0.507	0.001	0.013	0.043	0.005	0.032	0.001	-0.001	0.764	-0.008	-0.010	-0.010	READER NUMBER:
0.629	0.815	0.001	0.005	0.032	0.007	0.027	0.003	-0.001	1.544	-0.010	-0.010	-0.010	
0.801	0.829	0.005	0.008	0.021	0.012	0.017	0.001	-0.002	-0.002	-0.008	-0.010	-0.010	

CHOLESTEROL ASSAY											CALC
ug	OD 1	OD 2	MEAN	SD							STD
0	-0.003	0.003	0.000	0.004	m	b					-0.134
0.5	0.047	0.054	0.051	0.005	0.0783	0.0107	#N/A	#N/A	#N/A	#N/A	0.502
1	0.088	0.091	0.089	0.004	0.0013	0.0069	#N/A	#N/A	#N/A	#N/A	0.861
2	0.174	0.192	0.183	0.013	0.8884	0.0129	#N/A	#N/A	#N/A	#N/A	2.172
4	0.328	0.342	0.335	0.010	##	6.000	#N/A	#N/A	#N/A	#N/A	4.088
8	0.473	0.507	0.490	0.024	0.612	0.001	#N/A	#N/A	#N/A	#N/A	6.042
8	0.629	0.616	0.623	0.009							7.712
10	0.801	0.829	0.815	0.020							10.138



Author's Signature <i>Elaine Krul</i>	Date <i>12/2/96</i>	Read and Understood By <i>Denise Nachowiak</i>	Date <i>10-1-97</i>
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Book Number
GDS - 5734

Page
078

Subject
FPLC - Rabbit 08

Project Number
SEARLE

Project P
S

SAMPLE RESULTS (SINGULATES)

SAMP. NO.	(ml)	OD	CALC ug	CALC ug/ml	FXN VOL	ug FXN	OMIT (-)	TOTAL LIPID
1	0.060	-0.003	-0.172	-2.870	0.500	-1.435	0.000	31.448
2	0.060	0.003	-0.097	-1.810	0.500	-0.805	0.000	
3	0.060	0.005	-0.071	-1.180	0.500	-0.585	0.000	
4	0.060	-0.001	-0.147	-2.450	0.500	-1.225	0.000	
5	0.060	-0.001	-0.147	-2.450	0.500	-1.225	0.000	VLDL(5-13)
6	0.060	0.001	-0.122	-2.030	0.500	-1.015	0.000	0.035
7	0.060	0.001	-0.122	-2.030	0.500	-1.015	0.000	%
8	0.060	0.005	-0.071	-1.180	0.500	-0.585	0.000	0.11
9	0.060	0.009	-0.021	-0.349	0.500	-0.175	0.000	
10	0.060	0.011	0.004	0.071	0.500	0.035	0.035	
11	0.060	0.001	-0.122	-2.030	0.500	-1.015	0.000	
12	0.060	0.003	-0.097	-1.810	0.500	-0.805	0.000	
13	0.060	0.001	-0.122	-2.030	0.500	-1.015	0.000	
14	0.060	0.013	0.029	0.491	0.500	0.245	0.245	LDL(14-29)
15	0.060	0.005	-0.071	-1.180	0.500	-0.585	0.000	17.335
16	0.060	0.009	-0.021	-0.349	0.500	-0.175	0.000	%
17	0.060	0.013	0.029	0.491	0.500	0.245	0.245	55.12
18	0.060	0.02	0.118	1.981	0.500	0.981	0.981	
19	0.060	0.029	0.231	3.852	0.500	1.926	1.926	
20	0.060	0.039	0.357	5.953	0.500	2.976	2.976	
21	0.060	0.046	0.445	7.423	0.500	3.712	3.712	
22	0.060	0.043	0.408	6.793	0.500	3.396	3.396	
23	0.060	0.032	0.269	4.482	0.500	2.241	2.241	
24	0.060	0.021	0.130	2.171	0.500	1.086	1.086	
25	0.060	0.014	0.042	0.701	0.500	0.350	0.350	
26	0.060	0.011	0.004	0.071	0.500	0.035	0.035	
27	0.060	0.012	0.017	0.281	0.500	0.140	0.140	
28	0.060	0.005	-0.071	-1.180	0.500	-0.585	0.000	
29	0.060	0.003	-0.097	-1.810	0.500	-0.805	0.000	
30	0.060	0.005	-0.071	-1.180	0.500	-0.585	0.000	HDL(30-47)
31	0.060	0.007	-0.048	-0.770	0.500	-0.385	0.000	14.078
32	0.060	0.012	0.017	0.281	0.500	0.140	0.140	%
33	0.060	0.016	0.087	1.121	0.500	0.581	0.581	44.77
34	0.060	0.024	0.168	2.802	0.500	1.401	1.401	
35	0.060	0.031	0.256	4.272	0.500	2.136	2.136	
36	0.060	0.035	0.307	5.112	0.500	2.558	2.558	
37	0.060	0.038	0.319	5.322	0.500	2.681	2.681	
38	0.060	0.032	0.269	4.482	0.500	2.241	2.241	
39	0.060	0.027	0.208	3.432	0.500	1.716	1.716	
40	0.060	0.017	0.060	1.331	0.500	0.666	0.666	
41	0.060	0.001	-0.122	-2.030	0.500	-1.015	0.000	
42	0.060	0.005	-0.071	-1.180	0.500	-0.585	0.000	
43	0.060	0.005	-0.071	-1.180	0.500	-0.585	0.000	
44	0.060	0.005	-0.071	-1.180	0.500	-0.585	0.000	
45	0.060	0.001	-0.122	-2.030	0.500	-1.015	0.000	
46	0.060	0.001	-0.122	-2.030	0.500	-1.015	0.000	
47	0.060	0.003	-0.097	-1.810	0.500	-0.805	0.000	
48	0.060	0.001	-0.122	-2.030	0.500	-1.015	0.000	
49	0.060	0.001	-0.122	-2.030	0.500	-1.015	0.000	
50	0.060	-0.001	-0.147	-2.450	0.500	-1.225	0.000	
51	0.060	-0.001	-0.147	-2.450	0.500	-1.225	0.000	
52	0.060	0.001	-0.122	-2.030	0.500	-1.015	0.000	
53	0.060	0.001	-0.122	-2.030	0.500	-1.015	0.000	
54	0.060	-0.001	-0.147	-2.450	0.500	-1.225	0.000	
55	0.060	-0.001	-0.147	-2.450	0.500	-1.225	0.000	
56	0.060	-0.002	-0.180	-2.680	0.500	-1.330	0.000	
57	0.060	0.001	-0.122	-2.030	0.500	-1.015	0.000	
58	0.060	-0.003	-0.172	-2.870	0.500	-1.435	0.000	
59	0.060	-0.001	-0.147	-2.450	0.500	-1.225	0.000	
60	0.060	0.001	-0.122	-2.030	0.500	-1.015	0.000	
61	0.060	-0.001	-0.147	-2.450	0.500	-1.225	0.000	ACTUAL
Cardio1	0.060	0.784	0.784	12.733	10.000	127.333		194
Cardio2	0.060	1.544	1.544	25.733	10.000	257.333		381
	0.060	-0.002	-0.180	-2.680	10.000	-26.802		

Author's Signature
Diane Kuhl

Date
12/2/96

Read and Understood By
Denise Nachowick

Date
10-1-97

Author's
S

RLE

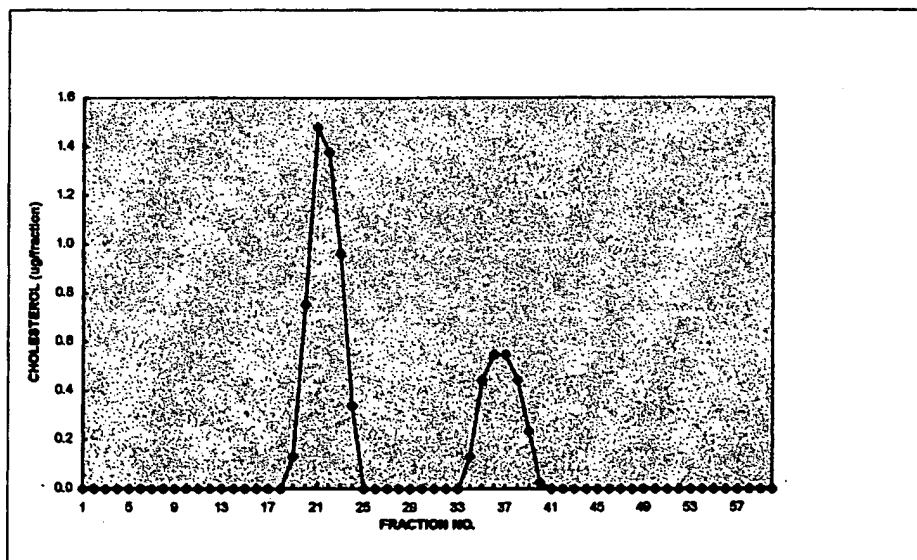
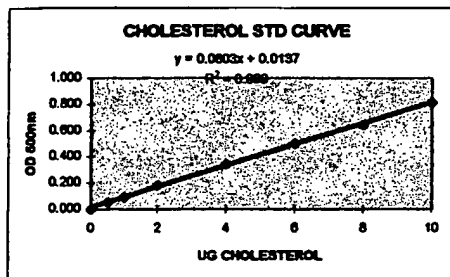
Project Number	Subject	Book Number
SEARLE	FPC - Rabbit 08	GDS - 5734
		Page
		079

	1	2	3	4	5	6	7	8	9	10	11	12
A	-0.001	0.001	0.001	0.006	0.009	0.013	0.010	0.010	0.003	0.003	-0.005	0.003
B	0.050	0.057	0.001	0.009	0.010	0.010	0.015	0.008	0.008	0.001	-0.005	-0.005
C	0.093	0.097	0.002	0.005	0.015	0.008	0.018	0.009	0.005	0.003	-0.005	-0.005
D	0.178	0.187	0.001	0.004	0.021	0.008	0.019	0.007	0.004	0.003	-0.005	-0.008
E	0.338	0.358	0.002	0.003	0.028	0.008	0.019	0.006	0.004	0.003	-0.004	-0.005
F	0.505	0.496	0.002	0.005	0.027	0.007	0.018	0.005	0.003	0.750	-0.005	-0.005
G	0.670	0.668	0.002	0.010	0.023	0.008	0.018	0.004	0.004	1.521	-0.005	-0.004
H	0.818	0.817	0.006	0.007	0.017	0.009	0.014	0.005	0.003	0.003	-0.004	-0.005

READ DATE:
11/1/86
ASSAY NAME:
PLATE NUMBER:
110198p2
READER NUMBER:

CHOLESTEROL ASSAY

ug	OD 1	OD 2	MEAN	SD								CALC
STD	OD 1	OD 2	OD	OD								STD
0	-0.001	0.001	0.000	0.001	m	b	#N/A	#N/A	#N/A	#N/A	#N/A	-0.171
0.5	0.050	0.057	0.054	0.005	0.0803	0.0137	#N/A	#N/A	#N/A	#N/A	#N/A	0.495
1	0.093	0.097	0.095	0.003	0.0010	0.0053	#N/A	#N/A	#N/A	#N/A	#N/A	1.012
2	0.178	0.187	0.183	0.008	0.9990	0.0100	#N/A	#N/A	#N/A	#N/A	#N/A	2.102
4	0.338	0.358	0.347	0.016	###	6.000	#N/A	#N/A	#N/A	#N/A	#N/A	4.151
6	0.505	0.496	0.501	0.008	0.627	0.001	#N/A	#N/A	#N/A	#N/A	#N/A	6.063
8	0.670	0.616	0.643	0.038								7.637
10	0.818	0.817	0.818	0.001								10.011



Author's Signature	Date	Read and Understood By	Date
Elaine Krul	12/2/86	Denise Nachowiak	10-1-97

RLE

Project Number	Subject	Book Number
SEARLE	Pocono Rabbits - CETP Immunization Results	GDS - 5734
		Page 083

Debbie Heuvelman organized the immunization of 1 goat and 2 rabbits with human recombinant CETP at Pocono Farms.

Summarized here are the results obtained by Annette Frick on the inhibitory activity of the purified IgGs from these animals.

See Annette Frick's notebook 6865 pp. 063 - for raw data.

From: ANNETTE G FRICK at MONSL708
Date: 9/23/96 11:25 AM
Priority: Normal
Receipt Requested
TO: ELAINE S KRUL at MONRCC02
CC: DEBORAH M HEUVELMAN
Subject: Pocono Antibodies

----- Message Contents -----

Hello!

I tested the nine antibodies in the two hour inhibition assay with rCETP:

Assay setup- made up 50µg 1ml stocks of each antibody; used TP2 as my control IgG; used rCETP prep 838 at a 1:300 dilution (which should yield ~20% transfer); used HDL146 & LDL149 as source of Lipoprotein pool.

-performed 1:2 serial dilutions 4x's of each antibody (50µg, 25µg, 12.5µg, 6.25µg, 3.125µg)

-IgG Wells: added 150µl Lipoprotein pool + 25µl IgG + 25µl CETP

-Control wells: added 150µl Lipo pool + 25µl CETP + 25µl Buffer

-Blanks: added 150µl of Lipo pool + 50µl Buffer

Results: the only antibody that inhibited was the control (TP2); I have attached the excel spread sheets (version 4.0) and also the graphs (Delta Graph).

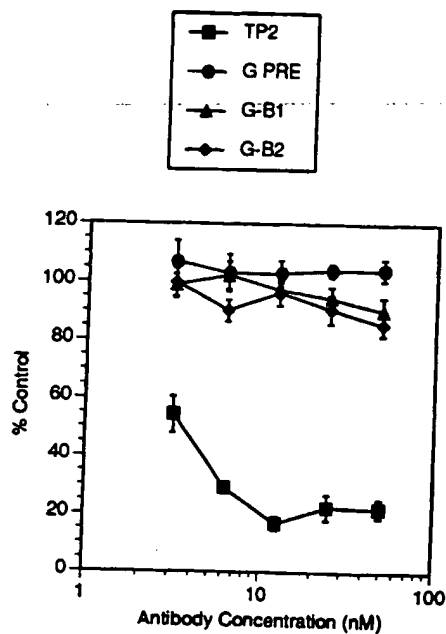
If you have any questions, please let me know. I will repeat the assays using the above conditions just to make sure the results are correct.

Annette

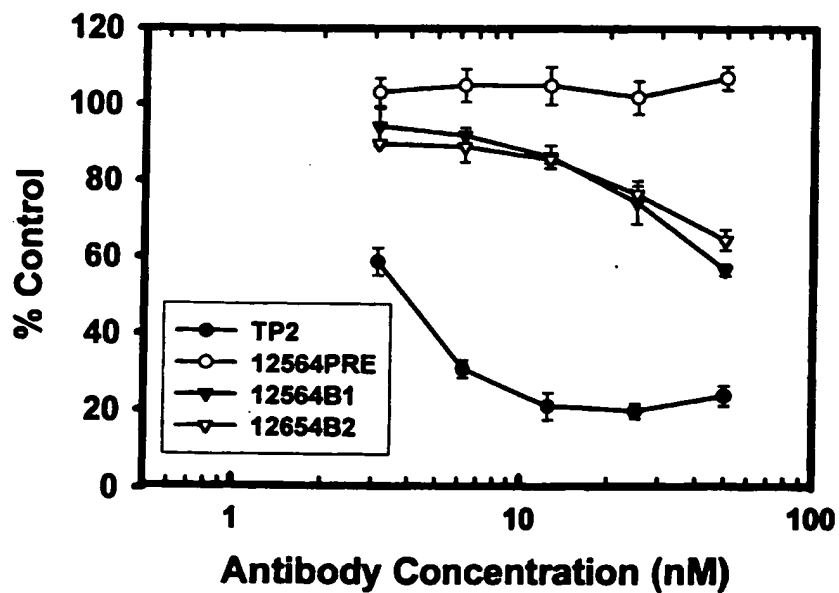
Author's Signature	Date	Read and Understood By	Date
Elaine Krul	1/24/97	Denise Nachowiak	10-1-97

-1-97

Book Number GDS - 5734	Subject <i>Pocono Rabbit - CETP Immunizations</i>	Project Number
Page 084		SEARLE



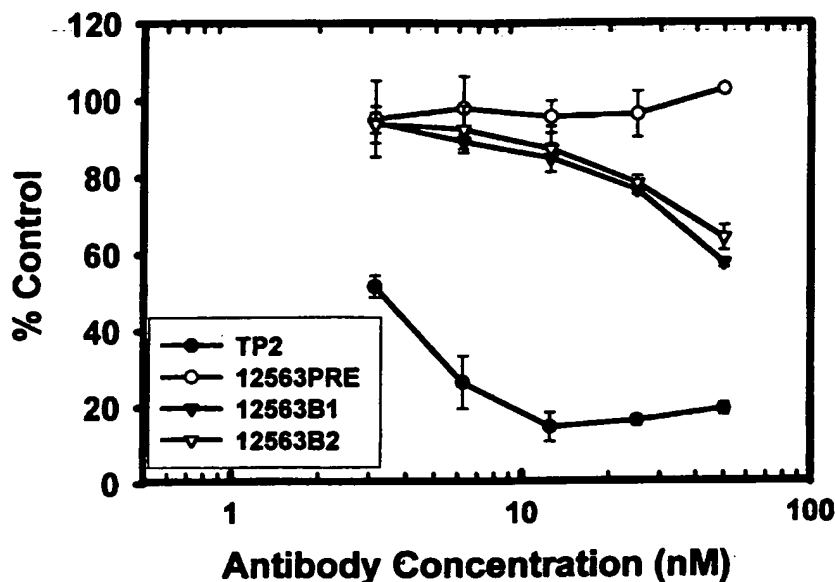
Pocono IgG 9-19-96



Author's Signature <i>Blaine Krul</i>	Date <i>1/24/97</i>	Read and Understood By <i>Denise Nachowiak</i>	Date <i>10-1-97</i>
--	------------------------	---	------------------------

Project Number	Subject	Book Number
SEARLE	Pocono Rabbit - CETP Immunizations	GDS - 5734
		Page
		085

Pocono IgG 9-19-96



Conclusions: I did not really look carefully at the initial data from Annette. It is true that the goat anti-human CETP antibody did not appear to inhibit at the doses tested compared to monoclonal TP2.

However, both rabbits showed inhibition of CETP activity. The IC_{50} of TP2 is ~2-4 nM and that of the polyclonal rabbit appears to be ~100 nM in our CETP assay.

Need to determine whether the IgG would inhibit rabbit CETP, because we could possibly treat rabbits passively to this IgG to inhibit CETP over a longer term.

Author's Signature	Date	Read and Understood By	Date
Elaine Kuhl	1/24/97	Denise Nachowiak	10-1-97

Project Number 565711	Subject TB2	Book Number GDS - 5748
SEARLE	1 week 1% Chol Diet	Page 125

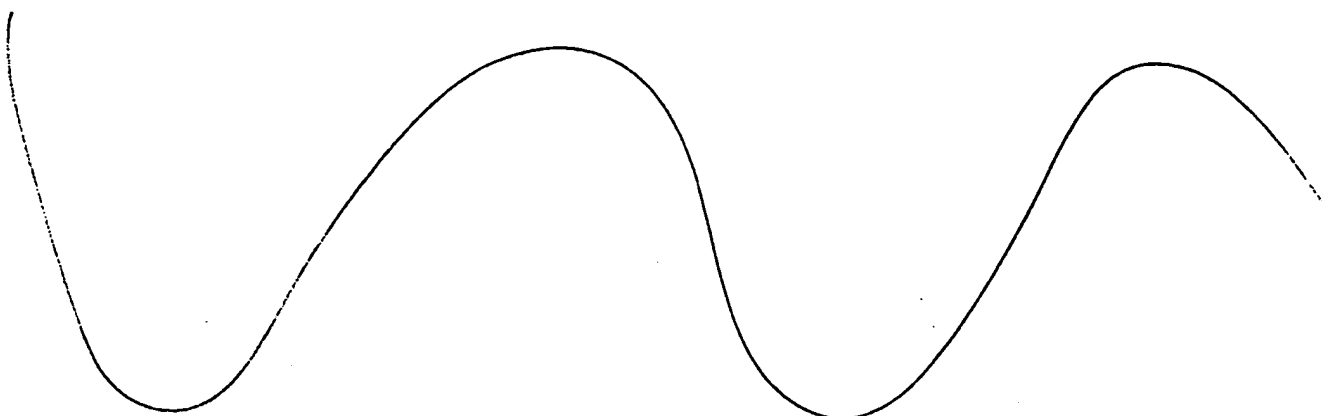
10/31 Compare CETP-TG and Non-Tg mice in Teklad 7001 w/ 1% cholesterol diet for 1 week (Begin diet on 10/25) Retro-orbital inject of HDL 151, p. 95, 50µl. Cardiac stick, under CO₂. 5 min + 24 hour time pt. Look at total cholesterol, FPLC cholesterol & counts.

12 Non-Tg mice $\begin{matrix} \swarrow 6 \\ \searrow 6 \end{matrix}$ 5 min group A
24 H group B

Reg # 12851
Lot # 1009960902
DOB 6-1-96

12 Tg mice $\begin{matrix} \swarrow 6 \\ \searrow 6 \end{matrix}$ 5 min group C
24 H group D

♂ 215, 216, 217, 218, 227, 224
♀ 219, 220, 221, ~~222~~ 213, 214
DOB 7/6, 6/17, 7/24



Author's Signature Beverly Kerce	Date 1-1-96	Read and Understood By G. Robinson	Date 1/27/99
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Book Number GDS - 5748	Subject T6 2, crit	Project Number 565711
Page 126		SEARLE

Proj

Animal weights

10/31

Group A
Non-Tq 5 min

A1	32.2 g	♂
A2	29.7	♂
A3	33.6	♂
A4	30.6	♀
A5	31.6	♀
A6	33.4	♀

Group B
Non-Tq 24 H.

B1	30.5	♂
B2	31.1	♂
B3	31.3	♂
B4	31.5	♀
B5	27.6	♀
B6	25.7	♀

*Deformed

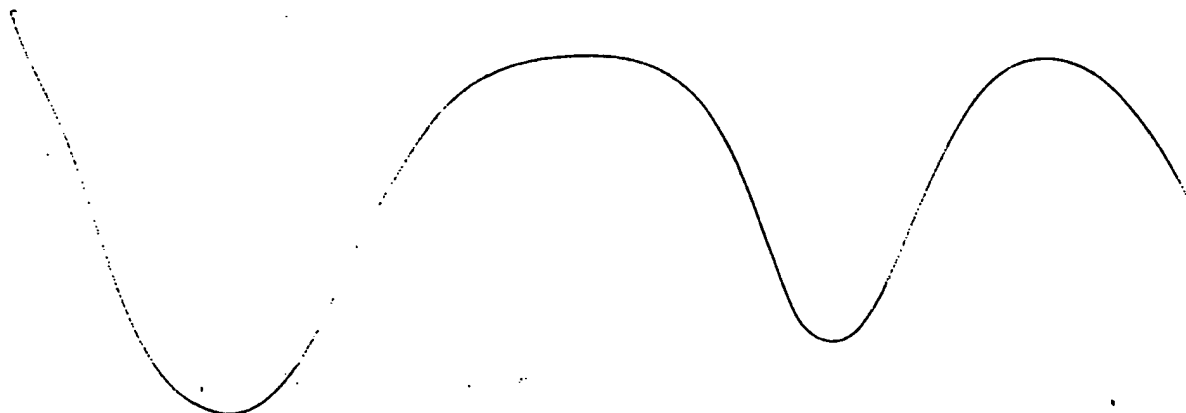
Group C
Tq 5 min

C1	26.8 g	♂
C2	25.3	♂
C3	26.7	♂
C4	22.0	♀
C5	23.3	♀
C6	23.6	♀

Group D
Tq 24 H.

D1	25.8	♂
D2	30.0	♂
D3	27.7	♂
D4	22.6	♀
D5	23.1	♀
D6	23.8	♀

♂ 27.97
Bk



W
R
C
L

Author's Signature <i>Bruce Keene</i>	Date <i>1/1/96</i>	Read and Understood By <i>[Signature]</i>	Date <i>1/27/99</i>
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Author's <i>[Signature]</i>

Project Number 505711	Subject TG2, cont.	Book Number GDS-5748
SEARLE		Page 127

10/31 and 1/1 DO cardiac stick for sampling. 26 gauge 1/2" medel. EDTA microtainer. DM use.
Spin. Save serum. Count 10 μ sera in 1 ml micro scint 20,
24 well plate.

DATA FILE NAME: FPLC.037
Save Each Plate to a File: no

10/31

5 min

CPM A	TSIS	
123.40	24.545	Blank
58.62	21.385	
91.60	19.682	
87.88	30.944	
152.30	24.258	
60.22	22.650	
6224.66	20.934	A1
3918.40	21.024	A2
4651.78	20.716	A3
4212.18	20.078	A4
4173.52	19.779	A5
4368.78	21.179	A6
6119.66	20.682	C1
7146.76	19.102	C2
5374.58	20.895	C3
7958.26	20.790	C4
1834.62	20.186	C5
6280.58	21.601	C6
50.56	25.955	Blank
36.14	19.621	
44.48	23.211	
55.24	27.716	
67.12	19.764	
34.20	22.627	

1/1

24h

CPM A	TSIS	
314.76	22.120	B1 10 μ
240.92	21.641	B2
233.92	21.016	B3
201.12	23.506	B4
321.48	23.212	B5
414.90	21.229	B6
233.54	21.842	D1
264.34	22.234	D2
223.68	24.461	D3
239.38	21.270	D4
250.92	25.469	D5
186.68	20.697	D6
41.88	36.843	Blank
29.02	21.416	
36.56	30.003	

Use 200 μ each to make pools. 1200 μ total/group. Filter.
Pipette 500 μ to FPLC. (Superose 6 x2)
Count 200 μ / fx in 1 ml scint 20
Do cholesterol profiles for fx's.

Author's Signature <i>Barry Keene</i>	Date 11-5-96	Read and Understood By <i>Robyn</i>	Date 1/27/99
--	-----------------	--	-----------------

Book Number GDS - 5748	Subject TG2, cont.	Project Number 565711
Page 128		SEARLE

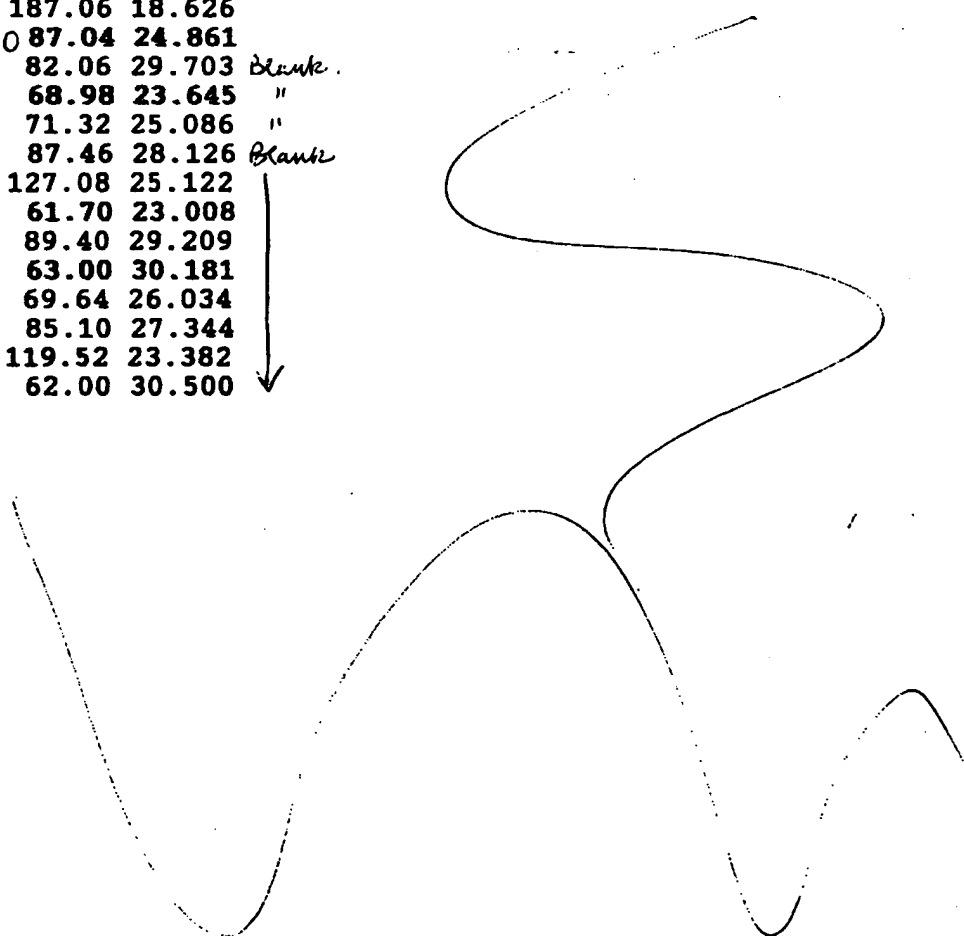
FPLC037.XLS

Non-Tg 5min		01 Nov 96 13:40	A
1	239.88 21.347	CPM A	tSIS
	141.16 19.385		
	215.12 12.974	49	195.96 21.507
	204.70 22.249		136.30 21.854
	382.96 17.688		161.46 18.403
	252.40 17.550		140.94 23.046
	453.54 19.964		207.04 19.124
	331.86 19.364		102.06 24.766
	288.76 18.095		132.58 24.636
10	254.00 20.633		94.66 28.684
	349.88 20.042		110.60 24.419
	191.24 18.292		134.08 25.568
	240.36 21.545		187.06 18.626
	196.54 20.314	60	87.04 24.861
	247.78 18.812		82.06 29.703
	265.24 19.297		68.98 23.645
	398.86 18.202		71.32 25.086
	269.68 18.754		87.46 28.126
	361.00 20.812		127.08 25.122
30	328.32 19.718		61.70 23.008
	361.04 19.103		89.40 29.209
	383.78 19.584		63.00 30.181
	450.32 17.651		69.64 26.034
	299.70 18.316		85.10 27.344
	283.70 19.889		119.52 23.382
	253.72 18.245		62.00 30.500
	305.32 18.926		
	404.74 20.044		
	634.12 17.430		
50	889.14 18.614		
	1604.98 18.621		
	3009.00 18.156		
	5111.74 18.608		
	6682.70 17.738		
	8224.08 16.785		
	11915.0 18.110		
	13994.4 17.948		
	11286.4 18.008		
	7128.88 18.744		
40	4095.04 18.430		
	2155.02 17.664		
	1341.28 18.533		
	823.46 19.024		
	538.90 18.430		
	338.28 18.171		
	260.64 19.023		
	261.82 18.733		
48	142.76 18.666		

GROUP B FPLC038.XLS
NON-TG 24 H

GROUP C FPLC039.XLS
TG 5 MIN

GROUP D FPLC040.XLS
TG 24 H



Author's Signature <i>Donalyn Kerce</i>	Date <i>11-25-96</i>	Read and Understood By <i>[Signature]</i>	Date <i>1/27/99</i>
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Project

Author
<i>E</i>

Project Number 565711	Subject TG2, cont.	Book Number GDS-5748
SEARLE		Page 129

GROUP D FPLC 040. XLS
TE 24H

CPM A tSIS
79.72 27.230
37.88 33.513
41.68 40.009
54.78 31.989
70.94 24.300
101.12 23.576
179.36 21.051
158.36 21.395
112.56 21.648
100.50 25.053-10
88.62 28.486
70.94 26.321
88.34 29.045
80.38 28.029
89.46 23.081
106.68 28.913
125.14 23.723
106.48 21.804
129.56 23.944
123.10 22.680-30
124.46 24.326
129.26 23.217
111.08 23.174
78.44 26.358
88.32 32.112
69.48 29.114
67.14 29.255
81.62 30.162
97.72 23.871
78.64 24.628-50
114.76 23.151
122.54 24.311
147.80 20.301
168.20 21.131
198.06 19.326
253.28 19.703
321.66 19.672
327.14 19.425
240.92 20.739 39

01 Nov 96 22:17 TopCount - 3.01

CPM A tSIS
184.12 21.468 40
141.84 21.307
115.12 23.792
104.46 24.942
88.14 21.722
81.34 25.328
90.98 29.855
117.30 24.722
66.08 32.312
73.18 27.715 Blank
59.26 31.939 49
59.62 42.027 50
63.98 27.809
72.34 37.876
44.78 37.042
50.16 34.145
44.20 29.876
55.80 29.434
56.36 28.807
78.20 32.987
54.52 35.825
56.12 33.481 60
39.84 32.848 Buffer (200µl)
42.72 40.845 "
48.60 34.293 "
29156.5 12.142 300µl pool A
1316.74 12.845 " B
39106.1 12.210 " C
1168.92 12.939 " D
47.38 37.041 Blank
61.46 32.212
82.94 35.925
45.22 32.732

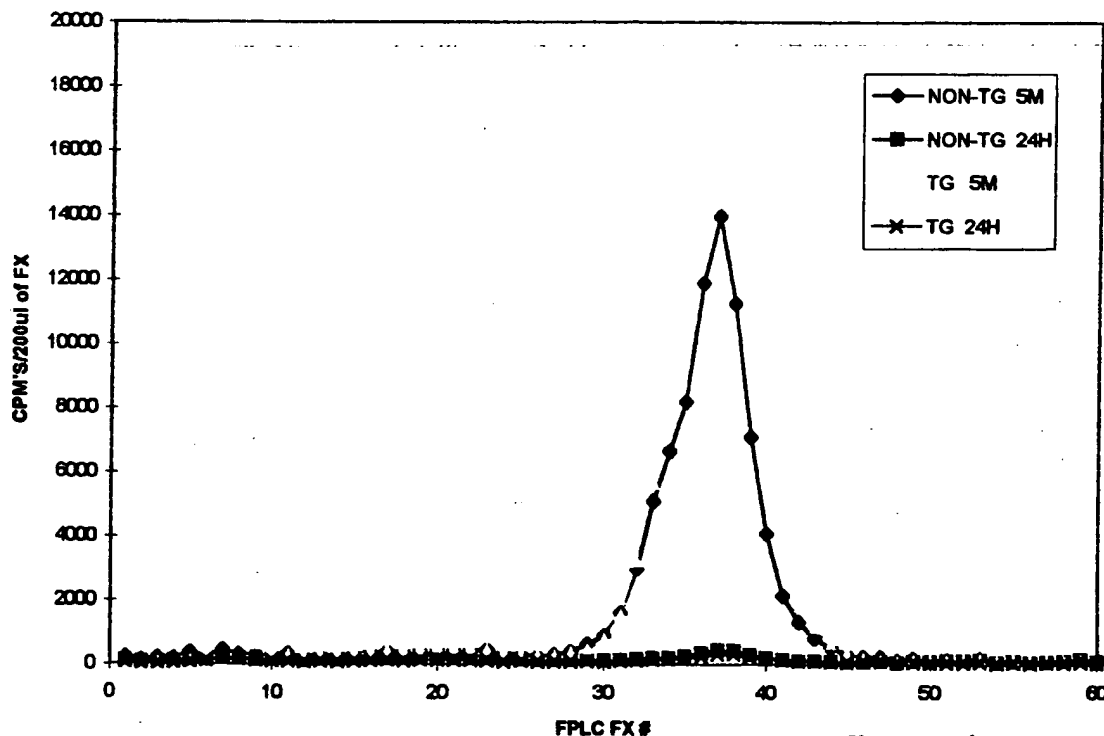
1-60
SUM OF CPIN'S
90483
10068
117664
6468

Author's Signature <i>Beverly Kepec</i>	Date 11-5-96	Read and Understood By <i>Robinson</i>	Date 1/27/89
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Book Number GDS - 5748	Subject TG-2, cont	Project Number 565711
Page 130		SEARLE

Project No. S

1% CHOL DIET 1 WEEK FPLC COUNTS



FPLC037.XLS

The counts are gone after 24 hours.

Next experiment will go for 4 hours.

[Handwritten wavy line]

Author's Signature L. Murrey-Kelley	Date 11-5-96	Read and Understood By [Signature]	Date 1/27/99
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Author's [Signature]

Project Number 505711	Subject TB2, cont.	Book Number GDS - 5748
SEARLE		Page 131

DATE: 11-1-96												
ASSAY: TCHOL												
	1	2	3	4	5	6	7	8	9	10	11	12
A	C	D	A1		B3		C5		A			
B	0.5		A2		B4		C6		B			
C	1		A3		B5		D1		C			
D	2		A4		B6		D2		D			
E	4		A5		C1		D3		CON			
F	6		A6		C2		D4		CON			
G	8		B1		C3		D5		2			
H	10		B2		C4		D6					

Samples 1:10 use 40ul + 60ul H₂O
(30ul water + 20ul TB2)

Control 1:20 use 40ul + 40ul H₂O
(20ul water + 20ul TB2)

TOTAL CHOLESTEROL					
11/4/86					
	NON-TG	NON-TG	TG	TG	
	5 MIN	24 H	5 MIN	24 H	
	GROUP A	GROUP B	GROUP C	GROUP D	
	78.4	89.8	71.2	85.9	
	66.4	84.9	75.2	83.5	
	69.2		78.2	58.2	
	62.3	48.8	62.1	58.7	
	69.0	68.8	53.3	22.8	
	66.8	58.5	68.4	58.5	
	MEAN	68.5	71.7	67.7	57.9
	STDEV	7.1	20.6	8.7	19.7

TG-2TCH.XLS

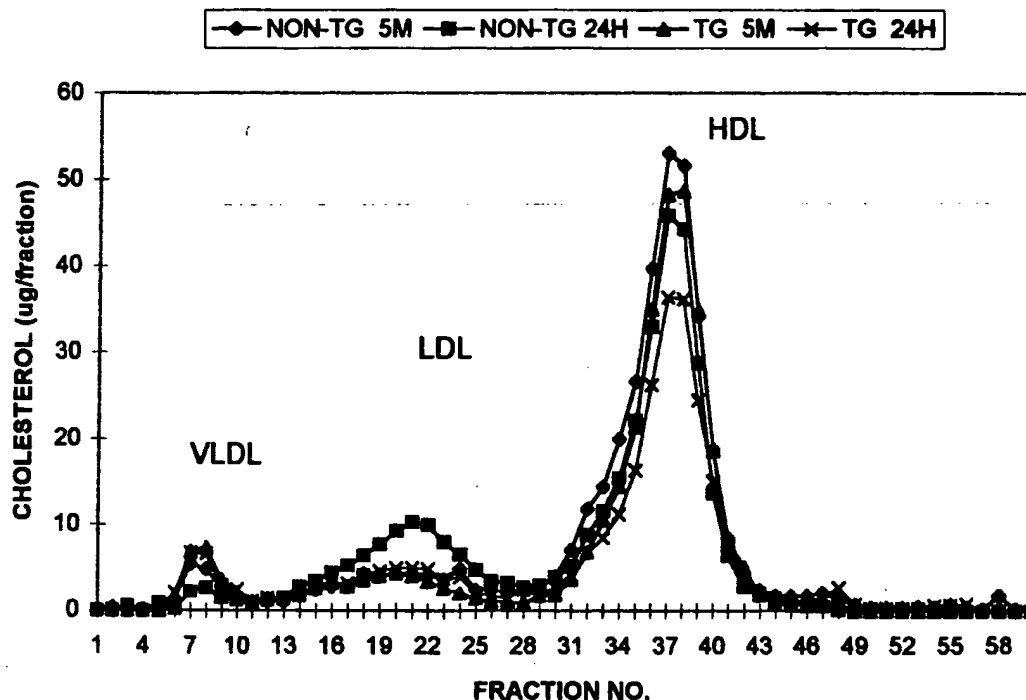
Total cholesterol show no change between B and Non-Tg or between 5 min and 24 hour.

TCHOL
BK12749

Author's Signature Barbara Kerce	Date 11-5-96	Read and Understood By [Signature]	Date 1/27/99
--	------------------------	--	------------------------

Book Number GDS - 5748	Subject TG2	Project Number 505711
Page 132	1% CHOL DIET 1 WEEK	SEARLE

Project A
S



TG2					
11/4/96					
1 WEEK 1% CHOL DIET					
FPLC PROFILE SUMMARY					
	NON-TG	NON-TG	TG	TG	
	6 MIN	24 H	6 MIN	24 H	
	GRP A	GRP B	GRP C	GRP D	
VLDL fx 5-13	16.2	8.7	21.6	21.2	
%	4	3	7	7	
LDL fx 14-29	52.3	91.8	41.1	51.8	
%	14	26	12	18	
HDL fx 30-47	304.7	247.9	263.6	207.2	
%	79	70	80	71	
TOTAL ug CHOL	385.4	352.7	330.9	291.7	

VLDL is up compare to previous exp. (on regular chow)
 LDL up slightly
 HDL is the same. see pg. 111

Author's Signature <i>Bruce K. Klee</i>	Date <i>11-5-96</i>	Read and Understood By <i>B. Klee</i>	Date <i>1/27/99</i>
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Author's <i>B</i>

Project Number 565711	Subject TG3	Book Number GDS - 5748
SEARLE	1% CHOLESTEROL CHOW/2 WEEK	Page 133

11-6 12 mice (6 Tg and 6 Non-Tg) on 1% chol. diet since 10/25 (12 days).

Micki Melton will inj 50µl HDL 151, p. 95. via orbital sinus. 4 hour incubation. Under CO₂ bleed via cardiac stick. Use EDTA tubes, on ice

Group A Non-Tg	A1	♀	30.8 g.
	A2	♀	30.4
	A3	♀	30.0
	A4	♂	28.4
	A5	♂	28.7
	A6	♂	26.7

Group B CETP Tg	B1	♀	22.7 g.
	B2	♀	21.5
	B3	♀	22.8
	B4	♂	22.4
	B5	♂	25.8
	B6	♂	23.8

FS ♀ 231, 232 ^{DOB} 8-1-96
FS ♂ 241, 242, 243 ^{DOB} 8-9-96

FPLC 041	CPM A	tsis
	1238.54	21.029 A1
	607.96	20.839 A2
	1245.32	21.503 A3
	1737.68	21.216 A4
	1119.26	18.198 A5
	1582.14	20.580 A6
	693.54	17.554 B1
	752.00	18.205 B2
	825.46	20.082 B3
	1825.60	18.602 B4
	883.86	15.315 B5
	277.80	18.114 B6

10µl sera in 1 ml serum. 20

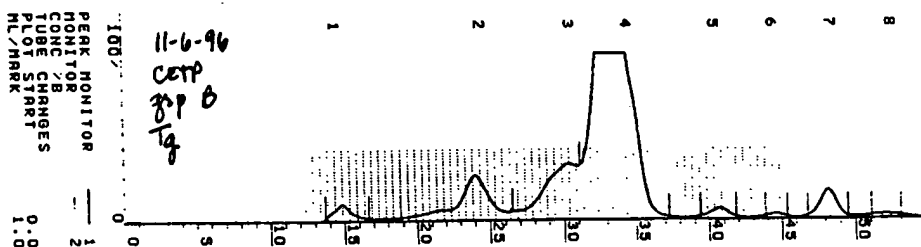
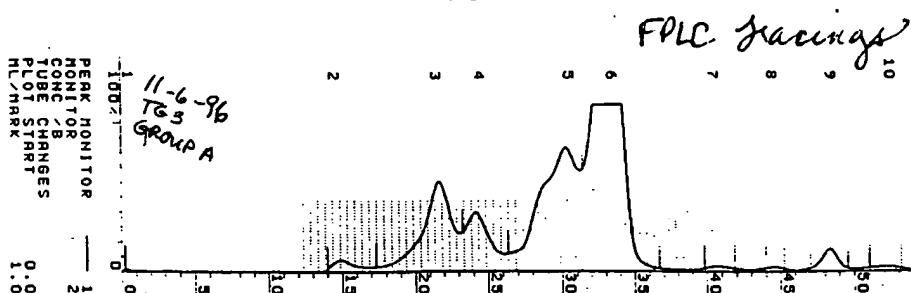
Author's Signature Beverly Kiker	Date 11-6-96	Read and Understood By PS Pohl	Date 1/27/99
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Book Number GDS - 5748	Subject TG3, cont.	Project Number 565711
Page 134		SEARLE

Project A
S

11-6-96 200 μ l each, Group A
fetter
500 μ l applied to FPLC

Group B, 135 μ l each
fetter
500 μ l applied



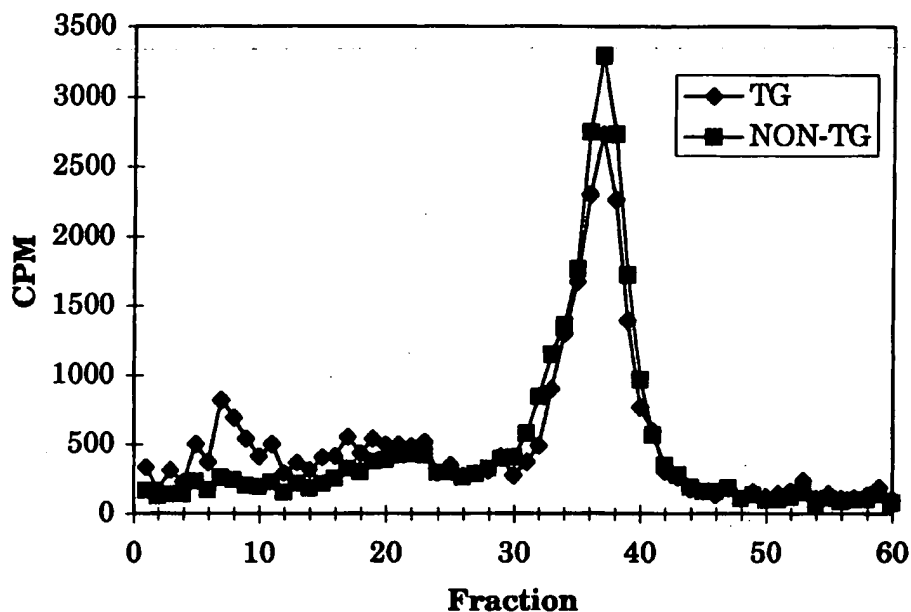
Count 200 μ l of each fx on top count, in 1 ml scint 20.
FPLC. 044 Group A - Tg
FPLC. 043 Group B - Non-Tg.

Author's Signature <i>B. K. K.</i>	Date 11-8-96	Read and Understood By <i>B. K. K.</i>	Date 1/27/99
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Author's <i>B. K. K.</i>

Project Number 505711	Subject TG3, cont	Book Number GDS - 5748
SEARLE		Page 135

4 Hour CETP Activity in vivo



	TG	NON-TG
VLDL	4488	1887
% of total	15	7
LDL	6586	5180
% of total	22	18
HDL	16245	19428
% of total	54	68
Total	30170	28449

Counts
% of Total

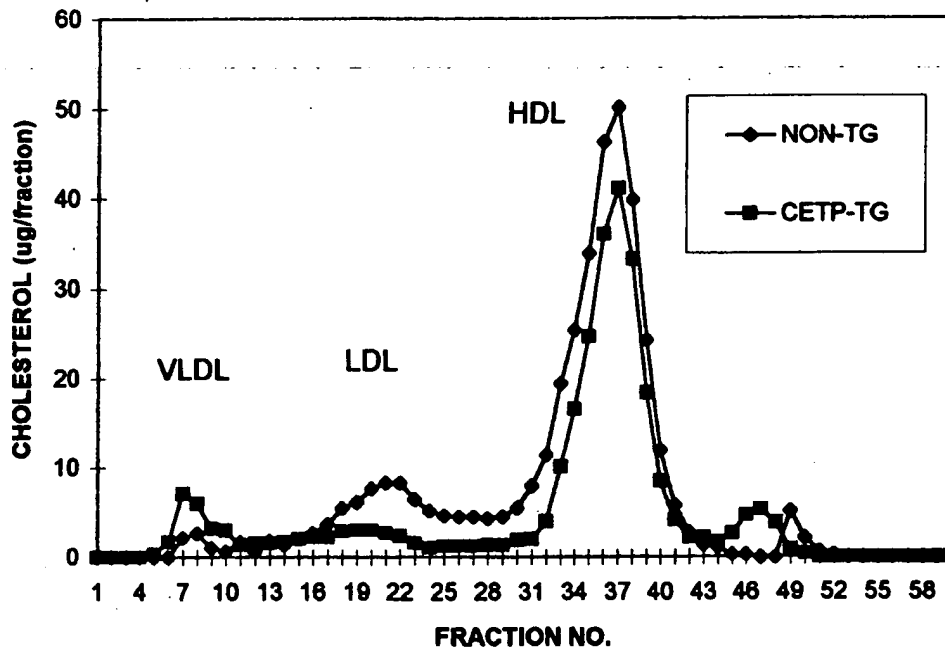
The Tg VLDL is up (15%). There may be some transfer activity going on. We may need to get total counts up even higher and get cholesterol up higher.

Author's Signature <i>Brucey Keker</i>	Date <i>11-8-96</i>	Read and Understood By <i>B. Robin</i>	Date <i>1/27/98</i>
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Book Number GDS - 5748	Subject TG3, cont. 2 WK diet/4 hour	Project Number 565711
Page 136		SEARLE

Project N S

2 WEEK DIET/4 HOUR 11-7-96



filename: TG3 Sum. XLS

TG3			
11/7/96			
2 WEEK 1% DIET			
4 HOUR			
		NON-TG	CETP-TG
		GRP A	GRP B
VLDL fx 6-13		6.6	21.5
%		2	8
LDL fx 14-29		79.9	31.2
%		21	11
HDL fx 30-47		288.2	219.9
%		74	78
T TAL ug CHOL		387.3	282.3

Author's Signature <i>Bruce Kepec</i>	Date <i>11-8-96</i>	Received Understood By <i>[Signature]</i>	Date <i>1/27/98</i>
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Author's S <i>[Signature]</i>

Project Number 565711	Subject TB3 2 week diet, 4 hour	Book Number GDS - 5748
SEARLE		Page 137

Lipid profile, p. 136, shows VLCL up to 8% from 1% (normal chow, p. 111)

Total cholesterol is not higher - we may need a longer time period or we may need additional fat in diet. Check literature.

TOTAL CHOLESTEROL			
11/7/96			
	NON-TG		CETP-TG
	4 HOUR		4 HOUR
	GROUP A		GROUP B
	99.8		85.6
	43.0		
	62.3		68.4
	91.7		88.5
	73.9		90.3
	104.4		
	MEAN	79.2	78.2
	STDEV	23.8	13.0

Author's Signature <i>Bruce Kline</i>	Date 11-8-96	Read and Understood By <i>[Signature]</i>	Date 1/27/98
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Book Number GDS - 5748	Subject <i>Lipoprotein Isolation</i>	Project Number <i>565711</i>
Page 138		SEARLE

Mouse Lipoproteins

Cholesterol (µg/ml)

Whole Sera		1181
	%	
% VLDL	5.9	69.7
% LDL	10.9	128.7
% HDL	65.0	767.7
VLDL fraction		539
LDL fraction		907
HDL fraction		11740

Hamster Lipoproteins

Cholesterol (µg/ml)

Whole Sera		923
	%	
% VLDL	16.8	155.1
% LDL	20.1	185.5
% HDL	47.2	435.7
VLDL fraction		846
LDL fraction		820
HDL fraction		7468

*from mouse & hamster lipoprotein isolation,
p. 124.*

Author's Signature <i>Bruce Keker</i>	Date <i>11-8-96</i>	Read and Understood By <i>[Signature]</i>	Date <i>1/27/91</i>
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Project Number 545711	Subject Rabbit Lipoprotein Isolation	Book Number GDS - 5748
SEARLE		Page 139

11-13-96

100 ml of pre-immune Rabbit sera obtained from Harriet Kurlander.

$$D = 1.020$$

Divide into 4 tubes 20 ml each. Add ~ 3 ml 1.006 EDTA Na.

Ultra-cfg. 48K 4° 24 H. no brake

The remaining 13 ml of sera - aliquot 250 μ l ea. Store -40°C.

11-14-96

1. Collect VLDL (top) into 50 ml conical. ~ 25 ml. Spin. 12.5 ml each + 10 ml 1.006 EDTA Na.

2. Discard middle.

3. Collect LDL/HDL (bottom). Black rubber stopper part of cap broke off during spin. Some little particles of it contaminated the bottom.

20.2 ml

$$D = 1.055$$

$$\begin{aligned} \text{grams of NaBr} &= 20.2 (1.063 - 1.055) / 1 - (0.2447 \times 1.063) \\ &= 20.2 (0.008 / 1 - 0.026) \\ &= 20.2 (0.008 / 0.74) \\ &= 0.218 \quad D = 1.062 \end{aligned}$$

Add 3 ml 1.006 EDTA Na

4. Spin VLDL and LDL/HDL 48K 24 hours 10°C.

Author's Signature <i>Beverly K. Klee</i>	Date 11-14-96	Read and Understood By <i>Bob</i>	Date 1/27/89
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Book Number GDS - 5748	Subject Rabbit LDL/HDL prep, cont.	Project Number 565711
Page 140		SEARLE

11-18-96

on ~~take out off the top & put in the~~ ^{11-27-99 OK}

<sup>2
OK
1-27-99</sup> [VLDL and LDL (top) / HDL (bottom) were removed from etgg. on 11/15.
VLDL stored 4°C
LDL/HDL, separated, stored 4°C.]

LDL $D = 1.008$ $Vol = 6.6 \text{ ml}$ protocol p. 115

$$\begin{aligned} V_2 &= 6.6 (1.008 - 1.020) / (1.020 - 1.006) \\ &= 6.6 (-0.012) / (0.014) \\ &= 6.6 (-0.000168) \\ &= -0.0011088 \quad \text{????} \end{aligned}$$

Consult Elaine: Add 15 ml of 1.006 sol. (wash)
Spin 48 K 10°C

HDL $D = 1.078$ $Vol. = 13.8 \text{ ml}$

$$\begin{aligned} \text{grams Na Br.} &= 13.8 (1.21 - 1.078) / 1 - (0.2447 \times 1.21) \\ &= 13.8 (0.132) / 1 - 0.296 \\ &= 1.822 / 0.704 \\ &= 2.588 \quad \text{Add this amount of Na Br.} \end{aligned}$$

Add 5 ml 1.21 sol.
 $D = 1.199$

Spin 48 K 10°C 43 Hours.

Author's Signature Beverly Kene	Date 11-19-96	Read and Understood By [Signature]	Date 1/27/99
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Project Number 565711	Subject TG4 3week/2 Hour	Book Number GDS - 5748
SEARLE		Page 141

11-15-96 6 Non-Tg Group A
 6 CETP-Tg Group B

Mice on 1% Chol. diet since 10/25. Inj. 3HDL 151
 (p. 95) 50µl via intraperitoneal.

TG # 200, 201, 222, 223, 235, 229, 230 ?

Group A	A1	♀	32.9g.	Group B	B1	♀	31.7g.
	A2	♀	29.6		B2	♀	23.1
	A3	♂	29.2		B3	♀	20.6
	A4	♂	26.1		B4	♂	23.8
	A5	♂	28.4		B5	♂	24.9
	A6	♂	27.7		B6	♂	26.7

At 5 min. do eye stick w/ capillary tube for counts:
 Spin. Count 10µl in 200µl sent to
 FPLC. 049

CPM A	tsis
2379.44	18.744A1
1663.72	19.121A2
5213.60	20.093A3
5314.74	18.808A4
1818.54	18.052A5
3389.66	18.922A6
4217.52	19.897B1
2097.76	20.046B2
6357.04	20.839B3
2691.96	20.141B4
4276.56	17.673B5
3851.26	19.878B6
3613.66	21.858

→ sol. of HDL 151 (1:10)

Author's Signature <i>Brian K. K...</i>	Date 11-19-96	Read and Understood By <i>[Signature]</i>	Date 1/27/99
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Book Number GDS - 5748	Subject TB4 - cont.	Project Number 565711
Page 142		SEARLE

Take Cardiac stick into EDTA microtainers
BR1.37.99

grp A 200µl sera each for pool
filter
500µl applied

grp B 200µl sera, except #3 100µl anal
#5 100µl
filter
500µl applied

11-18-96 For total cholesterol use 40µl of 1:10 sol.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.003	0.003	0.208	0.215	0.139	0.137	-0.011	-0.012	-0.014	-0.012	-0.013	-0.013
B	0.036	0.036	0.244	0.254	0.116	0.119	-0.012	-0.012	-0.013	-0.013	-0.013	-0.013
C	0.073	0.081	0.218	0.221	0.001	0.005	-0.011	-0.011	-0.013	-0.011	-0.012	-0.012
D	0.158	0.162	0.257	0.264	0.156	0.158	-0.012	-0.008	-0.013	-0.012	-0.012	-0.012
E	0.304	0.319	0.282	0.282	0.244	0.249	-0.011	-0.011	-0.011	-0.012	-0.011	-0.012
F	0.458	0.458	0.244	0.262	0.158	0.162	-0.001	-0.009	-0.010	-0.012	-0.012	-0.013
G	0.604	0.605	0.177	0.184	0.003	0.008	-0.005	-0.010	-0.008	-0.012	-0.011	-0.012
H	0.750	0.755	0.190	0.192	0.003	0.006	-0.011	-0.011	-0.011	-0.011	-0.011	-0.012

READ DATE:

11/18/96

ASSAY NAME:

TB4 TCHOL

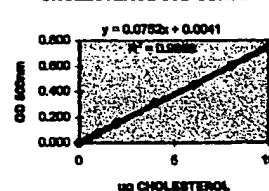
CHOLESTEROL ASSAY

ug	STD	OD 1	OD 2	MEAN	SD	OD	OD	mg/dl	CALC	STD
0	0.003	0.003	0.003	0.003	0.000					-0.014
0.5	0.036	0.036	0.036	0.036	0.001	0.0752	0.0041	#N/A	#N/A	0.418
1	0.073	0.081	0.077	0.077	0.008	0.0005	0.0025	#N/A	#N/A	0.970
2	0.158	0.162	0.160	0.160	0.003	0.8988	0.0048	#N/A	#N/A	2.074
4	0.304	0.319	0.312	0.312	0.011	#####	6.000	#N/A	#N/A	4.089
6	0.458	0.458	0.457	0.457	0.001	0.550	0.000	#N/A	#N/A	6.024
8	0.604	0.605	0.605	0.605	0.001			#N/A	#N/A	7.986
10	0.750	0.755	0.753	0.753	0.004			#N/A	#N/A	9.954

SAMPLE RESULTS (DUPLICATES)

SAMP.	NO.	(ml)	OD 1	OD 2	MEAN	SD	CALC.	CALC.	DF	mg/dl	
			OD	OD	OD	OD	ug	ug/ml		CHOL	
	1	0.040	0.208	0.215	0.212	0.005	2.759	68.970	10.000	68.970	A1
	2	0.040	0.244	0.254	0.249	0.007	3.258	81.439	10.000	81.439	A2
	3	0.040	0.218	0.221	0.220	0.002	2.865	71.630	10.000	71.630	A3
	4	0.040	0.257	0.264	0.261	0.005	3.410	85.282	10.000	85.282	A4
	5	0.040	0.282	0.282	0.282	0.000	3.696	92.411	10.000	92.411	A5
	6	0.040	0.244	0.262	0.253	0.013	3.311	82.769	10.000	82.769	A6
	7	0.040	0.177	0.184	0.181	0.005	2.347	58.683	10.000	58.683	B1
	8	0.040	0.190	0.192	0.191	0.001	2.486	62.154	10.000	62.154	B2
	9	0.040	0.139	0.137	0.138	0.001	1.781	44.532	10.000	44.532	B3
	10	0.040	0.116	0.119	0.118	0.002	1.509	37.716	10.000	37.716	B4
BLANK		0.040	0.001	0.005	0.003	0.003	-0.014	-0.355	10.000	-0.355	B5
	12	0.040	0.158	0.158	0.157	0.001	2.034	50.849	10.000	50.849	B6
	13	0.040	0.244	0.249	0.247	0.004	3.224	80.608	10.000	80.608	POOL A
	14	0.040	0.158	0.162	0.160	0.003	2.074	51.847	10.000	51.847	POOL B

CHOLESTEROL STD CURVE



filename: TB4 CH.XLS

Author's Signature <i>Bruce Kere</i>	Date <i>11-19-96</i>	Read and Understood By <i>[Signature]</i>	Date <i>1/27/97</i>
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50
45
40
35
30
25
20
15
10
5
0

CHOLESTEROL (ug/fraction)

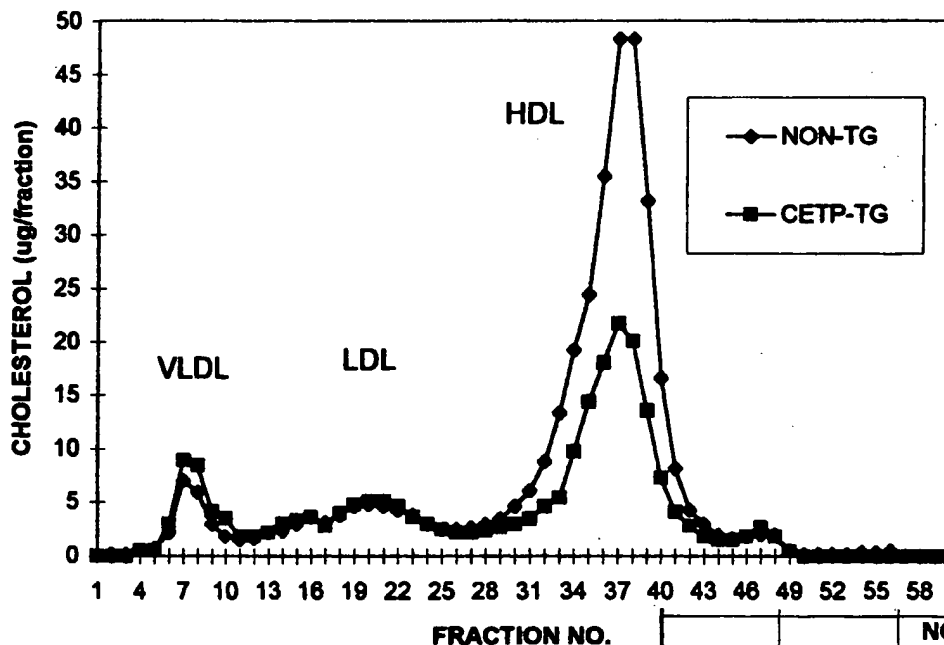
%
b
f

Author's
[Signature]

Project Number 565711	Subject TG4, cont.	Book Number GDS 5748
SEARLE		Page 143

TOTAL CHOLESTEROL			
3 WEEK DIET/2 HOUR			
11/15/86			
	NON-TG	CETP-TG	
	2 HOUR	2 HOUR	
	GROUP A	GROUP B	
	69.0	58.663	
	81.4	62.154	
	71.6	44.532	
	85.3	37.716	
	92.4		
	82.8	50.849	
	MEAN	80.4	50.8
	STDEV	8.7	10.0

TOTAL CHOLESTEROL 3 WEEK/2 HOUR



% of VLDL & LDL up
from 2 wk study, p. 136
in CETP-Tg mice.

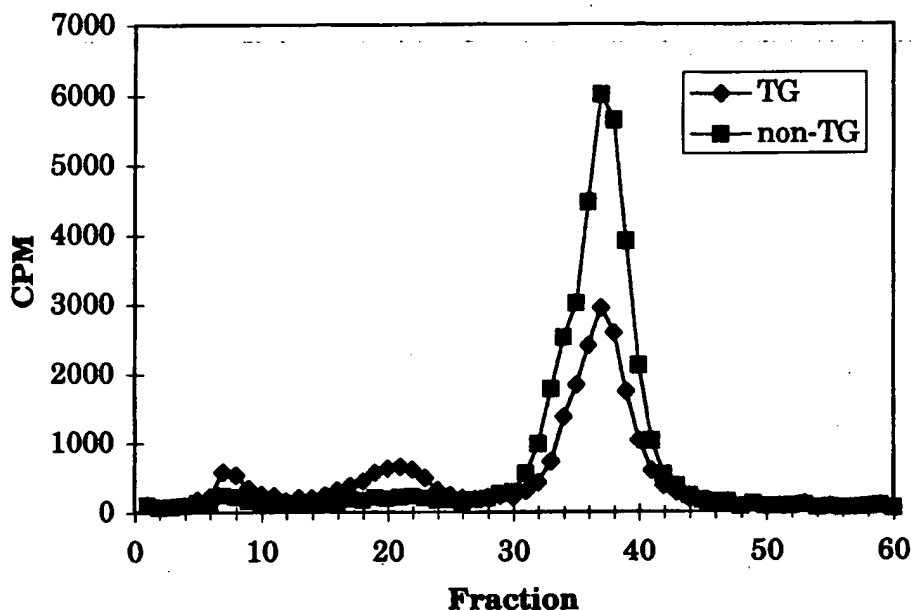
filename: TG4 SUM.XLS

	NON-TG	CETP-TG
	GRP A	GRP B
VLDL fx 5-13	20.8	28.6
%	6	12
LDL fx 14-29	55.3	55.1
%	15	24
HDL fx 30-47	281.7	138
%	76	60
TOTAL ug CH L	369.5	230.4

Author's Signature <i>Dorothy Klee</i>	Date 11-19-96	Read and Understood By <i>[Signature]</i>	Date 1/27/99
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Book Number GDS - 5748	Subject TB4 3wk/2 Hour	Project Number 505711
Page 144		SEARLE

2 Hour CETP Activity in vivo



We would like to get the counts up higher. M. Milton will dilute HDL 151 so we can inj. 100 μ l, raising counts, but not doubling which would be too many counts.

We will try a 4 week feeding study to stabilize levels.

Filename:

TB4 FPLCA = CETP-TG (GROUP B)

TB4 FPLCB.XLS = NON-TG (GROUP A)

Author's Signature <i>Beverly Keric</i>	Date <i>11-19-96</i>	Read and Understood By <i>Rob</i>	Date <i>1/27/98</i>
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Project Number 565711	Subject Rabbit HDL Prep, cont. from p. 140	Book Number GDS - 5748
SEARLE		Page 145

11-20-96

LDL There is no visible LDL layer on the bottom.
Collect top & middle together since they look
the same. Save 4°C.
Collect bottom (LDL) Save 4°C.

HDL Remove top (HDL). There is no middle. Save
bottom 4°C.

top: $D = 1.156$ Vol = 8.8 ml

$$\begin{aligned} V_2 &= 8.8 (1.156 - 1.020) / (1.020 - 1.006) \\ &= 8.8 (0.136) / (0.014) \\ &= 85.49 \text{ ml} \quad \text{Add this amount of 1.006 sol.} \end{aligned}$$

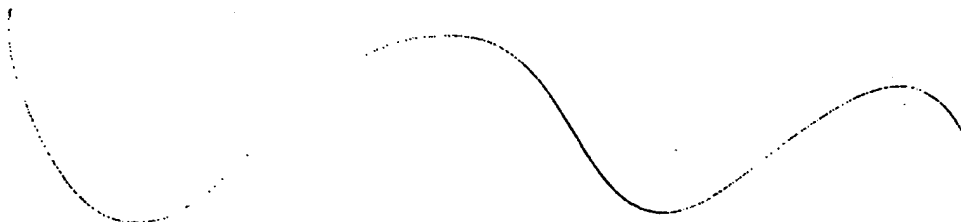
$$\begin{aligned} 85.49 + 8.8 &= 94.29 \text{ ml} \\ D &= 1.016 \end{aligned}$$

Split between 4 tubes. Drop one tube & lost
some.
48K 24H 10°C

11-21-96

HDL:
Remove top and middle. Save bottom (HDL)

$$D = 1.025 \quad \text{Vol} = 9.5 \text{ ml}$$



cont →

Author's Signature Beirley Kibee	Date 11-22-96	Read and Understood By [Signature]	Date 1/27/97
-------------------------------------	------------------	---------------------------------------	-----------------

Book Number GDS - 5748	Subject <i>Rabbit Lipoprotein Assay</i>	Project Number 565711
Page 146		SEARLE

1	2	3	4	5	6	7	8	9	10	11	12
0.002	0.002	0.193	0.224	0.078	0.072	0.022	0.018	0.038	0.040	-0.001	-0.001
0.031	0.031	0.015	0.018	0.003	0.008	0.868	0.858	1.428	1.322	0.002	-0.001
0.079	0.078	0.065	0.074	0.019	0.023	0.228	0.224	0.399	0.452	0.000	0.000
0.151	0.153	0.249	0.288	0.083	0.082	0.002	0.003	0.007	0.008	0.004	0.002
0.297	0.304	0.422	0.425	0.146	0.149	0.061	0.060	0.126	0.119	0.002	0.000
0.472	0.439	0.034	0.034	0.009	0.013	-0.002	0.001	0.000	-0.002	0.004	0.002
0.612	0.607	0.139	0.147	0.043	0.045	-0.002	-0.001	-0.002	-0.002	0.007	0.002
0.743	0.754	0.600	0.660	0.183	0.188	-0.002	0.001	-0.001	0.000	0.003	0.008

READ DATE:

11/22/96

ASSAY NAME:

Rabbit Lipoprotein

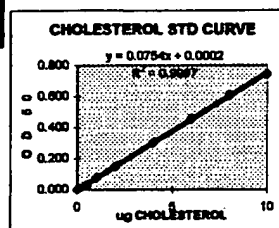
T Chol

CHOLESTEROL ASSAY

ug	STD	OD 1	OD 2	MEAN	SD	CALC	STD
0	0.002	0.002	0.002	0.000			0.024
0.5	0.031	0.031	0.031	0.000	0.0754	0.0002	0.408
1	0.079	0.078	0.079	0.001	0.0005	0.0026	1.038
2	0.151	0.153	0.152	0.001	0.9997	0.0048	2.013
4	0.297	0.304	0.301	0.005	6.000		3.981
6	0.472	0.439	0.456	0.023	0.553	0.000	6.036
8	0.612	0.607	0.610	0.004			8.078
10	0.743	0.754	0.749	0.008			9.921

SAMPLE RESULTS (DUPLICATES)

SAMP. NO.	(ml)	OD 1	OD 2	MEAN	SD	CALC.	CALC.	DF	mg/dl	CHOL
1	0.020	0.193	0.224	0.208	0.022	2.782	138.082	10.000	138.082	Sera
2	0.020	0.015	0.018	0.017	0.002	0.218	10.801	10.000	10.801	Idi
3	0.020	0.065	0.074	0.070	0.006	0.919	45.936	10.000	45.936	Idi
4	0.020	0.249	0.288	0.269	0.028	3.557	177.857	10.000	177.857	Idi
5	0.040	0.422	0.425	0.424	0.002	5.612	140.305	10.000	140.305	Sera
6	0.040	0.034	0.034	0.034	0.000	0.448	11.201	10.000	11.201	Idi
7	0.040	0.139	0.147	0.143	0.008	1.893	47.330	10.000	47.330	Idi
8	0.040	0.600	0.680	0.630	0.042	8.350	208.752	10.000	208.752	Idi
9	0.020	0.078	0.072	0.075	0.004	0.882	48.582	30.000	148.748	Sera
10	0.020	0.003	0.008	0.008	0.004	0.070	3.509	30.000	10.527	Idi
11	0.020	0.019	0.023	0.021	0.003	0.278	13.784	30.000	41.363	Idi
12	0.020	0.083	0.082	0.088	0.006	1.157	57.689	30.000	173.606	Idi
13	0.040	0.148	0.149	0.148	0.002	1.953	48.822	30.000	146.488	Sera
14	0.040	0.009	0.013	0.011	0.003	0.143	3.578	30.000	10.733	Idi
15	0.040	0.043	0.045	0.044	0.001	0.581	14.518	30.000	43.547	Idi
16	0.040	0.183	0.188	0.188	0.004	2.457	61.417	30.000	184.252	Idi
17	0.030	0.022	0.018	0.020	0.003	0.282	8.748	10.000	8.748	Idi top & mid
18	0.030	0.868	0.868	0.868	0.007	11.439	381.310	10.000	381.310	?????
19	0.030	0.228	0.224	0.226	0.003	2.994	99.789	10.000	99.789	Idi 11/20
20	0.030	0.002	0.003	0.003	0.001	0.030	1.013	10.000	1.013	Idi + 11/21
21	0.030	0.081	0.080	0.081	0.001	0.789	28.848	10.000	28.848	Idi-m 11/21
22	0.030	-0.002	0.001	-0.001	0.002	-0.009	-0.312	10.000	-0.312	blank
23	0.030	-0.002	-0.001	-0.002	0.001	-0.023	-0.754	10.000	-0.754	blank
24	0.030	-0.002	0.001	-0.001	0.002	-0.009	-0.312	10.000	-0.312	blank
25	0.080	0.038	0.040	0.039	0.001	0.514	8.572	10.000	8.572	Idi top & mid
26	0.080	1.428	1.322	1.374	0.074	18.214	303.573	10.000	303.573	?????
27	0.080	0.399	0.452	0.426	0.037	5.639	93.979	10.000	93.979	Idi 11/20
28	0.080	0.007	0.008	0.008	0.001	0.103	1.722	10.000	1.722	Idi + 11/21
29	0.080	0.128	0.119	0.123	0.005	1.821	27.024	10.000	27.024	Idi-m 11/21



	ug/ml	ml	TOTAL ug CHOL	% OF SERA
VLDL*	108.2	16	1731.2	1.5
LDL*	445.5	3.2	1425.6	1.2
HDL*	1881.2	9.8	18239.6	15.9
TOTAL			21396.4	18.6
LDL-top/mid	86.6	20	1732	1.5
????	3424.4	2	6848.8	6
HDL-b t (11/20)	968.8	10	9688	8.4
HDL-top (11/21)	13.7	29	397.3	0.3
HDL-mid (11/21)	268.4	43	11541.2	10.1
TOTAL			30207.3	26.3
SERA	1434	80	114720	

What happened to all the cholesterol??

Author's Signature <i>Bruce Kike</i>	Date <i>12-2-96</i>	Read and Understood By <i>Rob</i>	Date <i>1/27/99</i>
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Author's <i>1/27</i>

Project Number 505711	Subject CETP Activity Assay Rabbit CETP Vaccines	Book Number GDS - 5748
SEARLE		Page 147

67 samples from Elaine Kuhl 1:20 dilution in PBS (not assay buffer)

Lipo Pool LDL 149 10.84 mg/ml

$$\frac{200 \mu\text{g}/\text{ml}}{10840 \mu\text{g}/\text{ml}} = 0.0185 \times 18 \text{ ml} = 0.333 \text{ ml}$$

HDL 146 1.3 mg/ml

$$\frac{25 \mu\text{g}/\text{ml}}{1300 \mu\text{g}/\text{ml}} = 0.019 \times 18 \text{ ml} = 0.346 \text{ ml}$$

LDL 0.333 ml

HDL 0.346
0.679

$$18 - 0.679 = 17.321 \text{ assay buffer}$$

use 50 μl / well

positive control CETP 822 1:40
negative control Non-Tg mouse sera 1:5
samples 1:20

use 50 μl / well.

Follow protocol p. 10-11 overnight incubation

Author's Signature <i>Bruce K. Kice</i>	Date 12-2-96	Read and Understood By <i>BS/Koban</i>	Date 1/27/97
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Book Number GDS - 5748	Subject CETP Activity Rab. CETP Vaccine	Project Number 505711
Page 148		SEARLE

RAB SERA	PLATE 1									
CETP 617			BLANK	11723.2						
11/21/96										
		CPM	CPM	CPM	% T	% T	% T	AVE % T	ST DEV	
	BLANK	12668	11685.1	12020.2						
	BLANK	11329.2	11979.2	10114.5						
	BLANK	11486.2	11389.2	11668.2						
	BLANK	11558.7	12072.8	11696.1						
	BLANK	11893.8	11811.4	12879.1						
	BLANK	11795	10997.2	11976.5						
	POS	2065.56	1891.29	2495.94	82.38	83.87	78.71	81.65	2.65	
	NEG	11462.8	12032.6	12551	2.22	-2.64	-7.06	-2.49	4.64	
1	Rab01 pre 10/27/95	3875.9	3666.05	3398.83	66.94	68.73	71.01	68.89	2.04	
2	Rab01 12/4/95	3882.56	4256.04	3085.96	66.88	63.70	73.68	68.08	5.10	
3	Rab01 3/8/96	8349.12	8944.58	8303.97	28.78	23.70	29.17	27.22	3.06	
4	Rab02 pre 10/27/95	4581.58	4865.07	3981.44	60.92	58.50	66.21	61.88	3.94	
5	Rab02 12/4/95	4377.45	4494.86	4247.6	62.66	61.66	63.77	62.70	1.08	
6	Rab02 3/8/96	4618.67	4447.03	4734.88	60.60	62.07	59.61	60.76	1.24	
7	Rab02 10/25/96	4550.99	4086.02	4094.02	61.18	65.15	65.08	63.80	2.27	
8	Rab03 pre 10/27/95	4415.27	4823.48	4487.94	62.34	58.86	61.72	60.97	1.86	
9	Rab03 12/4/95	4307.01	4553.17	4959.84	63.26	61.18	57.69	60.70	2.81	
10	Rab04 pre 10/27/95	3555.2	3178.02	3762.43	69.67	72.89	67.81	70.16	2.53	
11	Rab04 12/4/95	3037.46	3073.36	3032.19	74.09	73.78	74.14	74.00	0.19	
12	Rab05 pre 10/27/95	3437.81	3648.62	3563.36	70.68	68.88	69.60	69.72	0.80	
13	Rab05 12/4/95	5400.6	5268.01	4801.46	53.93	55.06	59.04	56.01	2.68	
14	Rab06 pre 10/27/95	4242.56	3766.96	3427.64	63.81	67.87	70.78	67.48	3.49	
15	Rab06 12/4/95	3383.78	3501.59	3390.78	71.14	70.13	71.08	70.78	0.56	
16	Rab07 pre 10/27/95	4379.79	4159.54	3891.65	62.64	64.52	66.80	64.65	2.09	
17	Rab07 12/4/95	2788.94	3928.74	3735.38	76.21	66.50	68.14	70.28	5.20	
18	Rab07 3/8/96	3432.09	4473.96	4595.72	70.72	61.84	60.80	64.45	5.46	
19	Rab07 10/25/96	4413.87	4946.2	5412.43	62.35	57.81	53.83	58.00	4.28	
20	Rab08 pre 10/27/95	4184.52	4522.74	4928.46	64.31	61.42	57.98	61.23	3.17	
21	Rab08 12/4/95	3890.42	5312.94	4429.14	66.81	54.68	62.22	61.24	6.13	
22	Rab08 3/8/96	3824.96	5098.74	4409.04	67.37	56.52	62.39	62.10	5.43	
23	Rab08 10/25/96	5655.9	5653.7	6396.09	51.75	51.77	45.44	49.66	3.65	
24	Rab09 pre 10/27/95	2806.88	4412.42	3469.69	76.06	62.36	70.40	69.61	6.86	

Author's Signature <i>Bruce K. Kice</i>	Date <i>12-2-96</i>	Read and Understood By <i>[Signature]</i>	Date <i>1/27/99</i>
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Author's S
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Project Number 565711	Subject Cetp Activity	Book Number GDS - 5748
SEARLE	cont.	Page 149

RAB SERA	PLATE 2								
CETP617			BLANK	11542.0					
11/21/96									
		CPM	CPM	CPM	% T	% T	% T	AVE % T	ST DEV
	BLANK	11347.1	11022.3	11989					
	BLANK	12009.7	11456.3	11436.2					
	BLANK	12789.7	10995	11853.9					
	BLANK	11805.9	10728.8	11752.7					
	BLANK	11892	11180.9	11693.5					
	BLANK	11272.6	10781.4	11749.4					
	POS	1984.07	1737.95	2241.83	82.81	84.94	80.58	82.78	2.18
	NEG	11714.8	10221.3	13439.7	-1.50	11.44	-16.44	-2.17	13.95
25	Rab09 12/4/95	2876.7	3372.84	2784.53	75.08	70.78	75.87	73.91	2.74
26	Rab010 pre 10/27/95	3481.83	3825.55	3489.92	69.83	66.86	69.76	68.82	1.70
27	Rab010 12/4/95	3513.93	2634.1	2470.25	69.56	77.18	78.60	75.11	4.86
28	Rab1 pre 5/30/95	4122.71	3332.43	3644.45	64.28	71.13	68.42	67.94	3.45
29	Rab1 7/5/95	4280.54	3625.52	3844.06	62.91	68.59	66.70	66.07	2.89
30	Rab1 10/5/95	4447.04	4638.03	3626.82	61.47	59.82	68.58	63.29	4.65
31	Rab1 3/8/96	3863.95	4152.48	4086.49	66.52	64.02	64.59	65.05	1.31
32	Rab2 pre 5/30/95	3319.47	3094.2	3437.6	71.24	73.19	70.22	71.55	1.51
33	Rab2 7/5/95	3572	3856.23	4154.3	69.05	66.59	64.01	66.55	2.52
34	Rab2 10/5/95	3846.54	3924.51	4825.64	66.67	66.00	58.19	63.62	4.71
35	Rab2 3/8/96	4159.24	4448.53	4382.08	63.96	61.46	62.03	62.49	1.31
36	Rab3 pre 5/30/95	3839.38	4387.02	4324.64	66.74	61.99	62.53	63.75	2.60
37	Rab3 7/5/95	4115.6	4115.16	4189.82	64.34	64.35	63.70	64.13	0.37
38	Rab3 8/9/95	4352.96	4656.58	5002	62.29	59.66	56.66	59.53	2.81
39	Rab4 pre 5/30/95	3894.24	3518.18	4289.08	66.26	69.52	62.84	66.21	3.34
40	Rab4 7/5/95	3641.36	4282.17	3699.12	68.45	62.90	67.95	66.43	3.07
41	Rab4 8/9/95	3911.56	5721.82	4245.9	66.11	50.43	63.21	59.92	8.35
42	Rab5 pre 5/30/95	4170.85	5068.07	4585.3	63.86	56.09	60.27	60.08	3.89
43	Rab5 7/5/95	3664.33	4471.52	4706.89	68.25	61.26	59.22	62.91	4.74
44	Rab5 8/9/95	4652.84	5501.02	4763.51	59.69	52.34	58.73	56.92	3.99
45	Rab6 pre 5/30/95	4596.19	4913.98	4798.5	60.18	57.43	58.43	58.68	1.39
46	Rab6 7/5/95	3867.07	5507.57	4565.05	66.50	52.28	60.45	59.74	7.13
47	Rab6 8/9/95	3830.81	4305.69	5033.29	66.81	62.70	56.39	61.97	5.25
48	Rab7 pre 5/30/95	2868.99	3751.48	3494.88	75.14	67.50	69.72	70.79	3.93

Author's Signature <i>Beverly K. Keec</i>	Date <i>12-2-96</i>	Read and Understood By <i>[Signature]</i>	Date <i>1/27/99</i>
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Book Number GDS - 5748	Subject Rab. vaccine (CETP)	Project Number 565711
Page 150	CETP Activity	SEARLE

RAB SERA PLATE 3										
	CETP617			BLANK	11711.3					
	11/21/96									
		CPM	CPM	CPM	% T	% T	% T	AVE % T	ST DEV	
	BLANK	11566.9	11370.4	12201.8						
	BLANK	11384.8	11650.9	10820.9						
	BLANK	12289.2	11042.5	12221.4						
	BLANK	11225.7	11313.8	11772.6						
	BLANK	11350.8	11637.2	12781.4						
	BLANK	11139.5	12509.2	12524.9						
	POS	1897.2	2000.09	2516.98	83.80	82.92	78.51	81.74	2.84	
	NEG	11694.9	11904.4	11835.9	0.14	-1.65	-1.06	-0.86	0.91	
49	Rab7 7/5/95	4182	3731.66	3931.64	64.29	68.14	66.43	66.29	1.93	
50	Rab7 8/9/95	5435.63	3554.6	3861.19	53.59	69.65	67.03	63.42	8.62	
51	Rab8 pre 5/30/95	4857.51	4150.68	4684.63	58.52	64.56	60.00	61.03	3.15	
52	Rab8 7/5/95	5050.31	3949.26	4755.3	56.88	66.28	59.40	60.85	4.87	
53	Rab8 8/9/95	6240.13	4871.13	5092.99	46.72	58.41	56.51	53.88	6.27	
54	Rab9 pre 5/30/95	3564.33	3524.95	3166.44	69.57	69.90	72.96	70.81	1.87	
55	Rab9 7/5/95	4249.85	3850.43	3330.32	63.71	67.12	71.56	67.47	3.94	
56	Rab9 8/9/95	5141.1	5161.1	4539.72	56.10	55.93	61.24	57.76	3.02	
57	Rab10 pre 5/30/95	3699.63	4213.13	3900.3	68.41	64.03	66.70	66.38	2.21	
58	Rab10 7/5/95	3835.52	4467.33	4275.84	67.25	61.85	63.49	64.20	2.77	
59	Rab10 8/9/95	4466.25	5027.27	5166.34	61.86	57.07	55.89	58.27	3.16	
60	Rab11 pre 5/30/95	3547.52	3616.64	3568.83	69.71	69.12	69.53	69.45	0.30	
61	Rab11 7/5/95	4189.18	4020.36	4084.37	64.23	65.67	65.12	65.01	0.73	
62	Rab11 10/5/95	4274.55	4582.15	4306.03	63.50	60.87	63.23	62.54	1.45	
63	Rab11 3/8/96	4690.97	4912.59	4273.73	59.95	58.05	63.51	60.50	2.77	
64	Rab12 pre 5/30/95	4965.01	4364.85	4419.14	57.61	62.73	62.27	60.87	2.83	
	XXXXXX	3777.11	18872.3	18283.3	67.75	-61.15	-56.12	-16.50	73.01	
	XXXXXX	3669.25	18744.9	17655	68.67	-60.06	-50.75	-14.05	71.79	
	XXXXXX	4254.16	20575.8	20256.2	63.67	-75.69	-72.96	-28.33	79.69	
65	Rab12 7/5/95	4171.08	5196.97	4444.92	64.38	55.62	62.05	60.68	4.54	
66	Rab12 10/5/95	4134.73	5422.2	5410.88	64.69	53.70	53.80	57.40	6.32	
67	Rab12 3/8/96	4672.65	4655.12	5305.19	60.10	60.25	54.70	58.35	3.16	
	XXXXXX	17926.3	20072.4	20994.1	-53.07	-71.39	-79.26	-67.91	13.44	
	XXXXXX	19278.5	20677.3	17893.3	-64.61	-76.56	-52.79	-64.65	11.89	

Pos & Neg controls look good.

NE may have reached a plateau since all the beads have similar activity. Try a 4 hour incubation

Author's Signature <i>Power/Kirby</i>	Date <i>12-2-96</i>	Read and Understood By <i>Robins</i>	Date <i>1/21/97</i>
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Author's
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Project Number 505711	Subject Repeat CETP activity on Rab. CETP Vaccine Sera.	Book Number GDS - 5748
SEARLE		Page 151

Repeat CETP activity assay on Rabbit sera, p. 147-150.
Do a 4 hour incubation of lipopool and 1:20 dilution of sera.

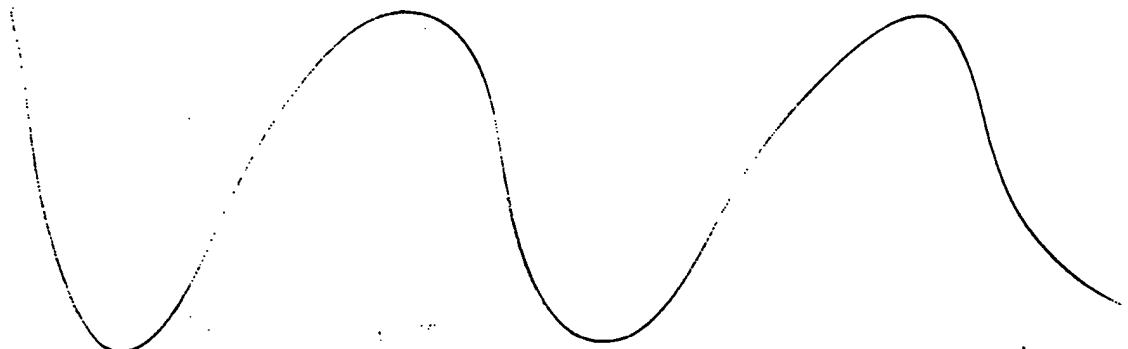
RAB SERA	PLATE 1								
CETP 618			BLANK	10610.5					
11/27/96									
		CPM	CPM	CPM	% T	% T	% T	AVE % T	ST DEV
	BLANK	10708.3	11038.6	10653					
	BLANK	10233.1	10087.6	10667.8					
	BLANK	11209.7	10967.8	11034.3					
	BLANK	10576.5	10234.2	11084.2					
	BLANK	10676.2	10625	10053.5					
	BLANK	10047.5	10189.6	10902.1					
	POS	2254.77	2740.44	2171.42	78.75	74.17	79.54	77.49	2.90
	NEG	9629.3	9864.26	9518.62	9.25	7.03	10.29	8.86	1.66
1	Rab01 pre 10/27/95	9012.24	9502.85	8241.26	15.06	10.44	22.33	15.94	5.99
2	Rab01 12/4/95	8259.51	8726.84	8249.08	22.16	17.75	22.26	20.72	2.57
3	Rab01 3/8/96	10566.2	9323.15	9536.1	0.42	12.13	10.13	7.56	6.27
4	Rab02 pre 10/27/95	10114.1	8181.17	8412.6	4.68	22.90	20.71	16.10	9.85
5	Rab02 12/4/95	10136	9739.08	8983.88	4.47	8.21	15.33	9.34	5.52
6	Rab02 3/8/96	9302.78	9212.79	8725.57	12.32	13.17	17.76	14.42	2.83
7	Rab02 10/25/96	8419.03	8191.19	8426.62	20.65	22.80	20.58	21.35	1.26
8	Rab03 pre 10/27/95	8792.16	8906.49	8196.75	17.14	16.06	22.75	18.65	3.59
9	Rab03 12/4/95	8743.68	9119.08	9092.86	17.59	14.06	14.30	15.32	1.98
10	Rab04 pre 10/27/95	8119.24	7743.19	8836.78	23.48	27.02	16.72	22.41	5.24
11	Rab04 12/4/95	8925.17	7544.31	8308.22	15.88	28.90	21.70	22.16	6.52
12	Rab05 pre 10/27/95	8381.97	7789.06	8647.97	21.00	26.59	18.50	22.03	4.14
13	Rab05 12/4/95	9813.49	9203.93	9662.6	7.51	13.26	8.93	8.80	2.99
14	Rab06 pre 10/27/95	9846.44	9315.92	9067.38	7.20	12.20	14.54	11.31	3.75
15	Rab06 12/4/95	9620.38	8990.1	8737.27	9.33	15.27	8.23	10.84	3.79
16	Rab07 pre 10/27/95	9309.37	8929.87	9379.61	12.26	15.84	11.60	13.23	2.28
17	Rab07 12/4/95	8378.32	8180.07	8082.44	21.04	23.09	24.01	22.72	1.52
18	Rab07 3/8/96	7948.98	8771.81	8647.07	25.08	17.33	18.50	20.31	4.18
19	Rab07 10/25/96	10046.1	10291.7	10192.9	5.32	3.00	3.94	4.09	1.16
20	Rab08 pre 10/27/95	9051.83	9461.79	9240.05	14.69	10.83	12.82	12.81	1.83
21	Rab08 12/4/95	9255.08	9036.05	9149.94	12.77	14.84	13.77	13.79	1.03
22	Rab08 3/8/96	8774.37	9884.1	8833.92	17.30	6.85	16.74	13.83	5.88
23	Rab08 10/25/96	9258.79	10007.7	9752.97	12.74	5.68	8.08	8.83	3.59
24	Rab09 pre 10/27/95	9322.28	9848.85	8886.41	12.14	7.17	16.25	11.85	4.55

Author's Signature <i>Beverly Kehee</i>	Date <i>12-2-96</i>	Read and Understood By <i>Bob Robison</i>	Date <i>1/27/99</i>
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Book Number GDS - 5748	Subject <i>cont.</i>	Project Number 565711
Page 152		SEARLE

Project
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	RAB SERA	PLATE 2								
	CETP618			BLANK	10503.0					
	11/27/96									
			CPM	CPM	CPM	% T	% T	% T	AVE % T	ST DEV
		BLANK	11282.2	10339.9	10494.8					
		BLANK	11017.4	11239.6	9986.81					
		BLANK	11006.6	11586.5	10983.2					
		BLANK	11114.8	10649.8	10276.2					
		BLANK	7210.34	10757.7	10340.8					
		BLANK	9808.3	10603.1	10356.1					
		POS	1973.51	2451.96	2474.61	81.21	76.65	76.44	78.10	2.69
		NEG	9809.02	10570.6	9869.69	6.61	-0.64	6.03	4.00	4.03
25	Rab09 12/4/95		9585.36	8612.11	8683.51	8.74	18.00	17.32	14.69	5.16
26	Rab010 pre 10/27/95		9191.15	8489.56	8887.09	12.49	19.17	15.39	15.68	3.35
27	Rab010 12/4/95		8071.4	7608.58	7770.91	23.15	27.56	26.01	25.57	2.24
28	Rab1 pre 5/30/95		8363.19	7508.84	8132.82	20.37	28.51	22.57	23.82	4.21
29	Rab1 7/5/95		9113.94	8269.4	8413.38	13.23	21.27	19.90	18.13	4.30
30	Rab1 10/5/95		8318.46	8156.34	8187.48	20.80	22.34	22.05	21.73	0.82
31	Rab1 3/8/96		10379.7	8945.67	9019.57	1.17	14.83	14.12	10.04	7.69
32	Rab2 pre 5/30/95		8298.45	8121.16	8340.71	20.99	22.68	20.59	21.42	1.11
33	Rab2 7/5/95		7370.99	8480.69	8384.95	29.82	19.25	20.17	23.08	5.85
34	Rab2 10/5/95		7666.33	8402.66	8714.38	27.01	20.00	17.03	21.35	5.12
35	Rab2 3/8/96		9285.39	9895.01	9315.07	11.59	5.79	11.31	9.56	3.27
36	Rab3 pre 5/30/95		9571.67	9752.46	9427.22	8.87	7.15	10.24	8.75	1.55
37	Rab3 7/5/95		9066.08	9559.74	9427.08	13.68	8.98	10.24	10.97	2.43
38	Rab3 8/9/95		8999.02	9667.69	8402.9	14.32	7.95	20.00	14.09	6.02
39	Rab4 pre 5/30/95		8019.45	8212.66	7808.4	23.65	21.81	25.66	23.70	1.93
40	Rab4 7/5/95		9453.04	9090.95	8065.25	10.00	13.44	23.21	15.55	6.85
41	Rab4 8/9/95		8089.22	9194.37	8746.71	22.98	12.46	16.72	17.39	5.29
42	Rab5 pre 5/30/95		8829.54	9717.02	8952.29	15.93	7.48	14.76	12.73	4.58
43	Rab5 7/5/95		8516.51	9987.64	9604.42	18.91	4.91	8.56	10.79	7.27
44	Rab5 8/9/95		9072.69	9874.18	9412.75	13.62	5.99	10.38	10.00	3.83
45	Rab6 pre 5/30/95		8469.02	9238.24	9653.13	19.37	12.04	8.09	13.17	5.72
46	Rab6 7/5/95		8991.33	9504.15	9788.33	14.39	9.51	6.80	10.24	3.85
47	Rab6 8/9/95		7722.64	8505.77	8486.19	26.47	19.02	19.20	21.56	4.25
48	Rab7 pre 5/30/95		8240.6	9020.81	8988.58	21.54	14.11	14.42	16.69	4.20



Author's Signature <i>Barney Kiper</i>	Date <i>12-2-96</i>	Read and Understood By <i>[Signature]</i>	Date <i>1/27/99</i>
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Author's <i>[Signature]</i>

Project Number 565711	Subject Cont.	Book Number GDS - 5748
SEARLE		Page 153

	RAB SERA	PLATE 3								
	CETP618			BLANK	10641.7					
	11/27/96									
			CPM	CPM	CPM	% T	% T	% T	AVE % T	ST DEV
		BLANK	11755	11175.2	11040.6					
		BLANK	10591.1	10163.1	11345.6					
		BLANK	10656.6	10384.9	11051.5					
		BLANK	9925.84	9460.2	10845.5					
		BLANK	10694.9	10054.4	10636.4					
		BLANK	10326.9	10346.4	11097.2					
		POS	2089.87	2422.13	2399.78	80.36	77.24	77.45	78.35	1.75
		NEG	10534.1	9918.48	9540.3	1.01	6.80	10.35	6.05	4.71
49	Rab7 7/5/95		9237.54	8913	8333.44	13.20	16.24	21.69	17.04	4.30
50	Rab7 8/9/95		8258.68	8941.53	8852.33	22.39	15.98	16.82	18.40	3.49
51	Rab8 pre 5/30/95		8512.38	8896.61	8121.48	20.01	16.40	23.68	20.03	3.64
52	Rab8 7/5/95		9413.3	8910.75	9439.19	11.54	16.27	11.30	13.04	2.80
53	Rab8 8/9/95		8694.87	8989.74	8716.37	18.29	15.52	18.09	17.30	1.54
54	Rab9 pre 5/30/95		7720.48	8551.09	7977.01	27.45	19.65	25.04	24.05	4.00
55	Rab9 7/5/95		7750.23	8723.74	9015.18	27.17	18.02	15.28	20.16	6.22
56	Rab9 8/9/95		8055.35	8291.32	7873.01	24.30	22.09	26.02	24.14	1.97
57	Rab10 pre 5/30/95		8579.67	7909.08	8875	19.38	25.68	16.60	20.55	4.65
58	Rab10 7/5/95		9027.55	8162.52	8941.2	15.17	23.30	15.98	18.15	4.48
59	Rab10 8/9/95		8578.41	8976.45	8962.59	19.39	15.65	15.78	16.94	2.12
60	Rab11 pre 5/30/95		8344.44	8058.18	8349.21	21.59	24.28	21.54	22.47	1.57
61	Rab11 7/5/95		9097.25	9635.55	9635.24	14.51	9.46	9.46	11.14	2.92
62	Rab11 10/5/95		9932.16	9223.43	9531.58	6.67	13.33	10.43	10.14	3.34
63	Rab11 3/8/96		10474.1	9505.89	9884.74	1.58	10.67	7.11	6.45	4.58
64	Rab12 pre 5/30/95		9460.02	8476.52	8583.1	11.10	20.35	19.34	16.93	5.07
65	Rab12 7/5/95		8598.62	10379.7	9104.66	19.20	2.46	14.44	12.04	8.62
66	Rab12 10/5/95		7502.73	9091.06	7972.8	29.50	14.57	25.08	23.05	7.67
67	Rab12 3/8/96		8929.18	10351.3	9343.77	16.09	2.73	12.20	10.34	6.87
			10594	11717	11683.6	0.45	-10.10	-9.79	-6.48	6.00
			10874.6	12001.5	11596.5	-2.19	-12.78	-8.97	-7.98	5.36
			9719.78	11477.5	10783.6	8.66	-7.85	-1.33	-0.17	8.32
			10828.9	11947	10791.4	-1.76	-12.27	-1.41	-5.14	6.17
			11358.3	12153.7	11568.4	-6.73	-14.21	-8.71	-9.88	3.87

There is much variability between replicates and no apparent CETP inhibition. The sera may not be stable in a 1:20 dilution - these are the same as previous assay (p. 148-150) This was a 4 hour incubation (previous assay was an overnight incubation) and may not have been long enough.

filename: CETP618

Author's Signature <i>Deverey Keene</i>	Date <i>12-2-96</i>	Read and Understood By <i>R. Johnson</i>	Date <i>1/27/99</i>
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7/99

Book Number GDS - 5748	Subject TG.5	Project Number 505711
Page 154	3 week / 5min - 2H - 4H	SEARLE

Nov. 27

18 **CETP-Tg** mice on 1% Chol diet 3 weeks

F5 # 271, 272, 273, 274, 275, 276, 277, 278,
279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291,
292, 293

Inj. 100 μ l of HDL 151 (diluted) via retro-orbital
sinus. After 5 min, 2 hour, 4 hour do cardiac
stick.

Group A 5 min

A1	♀	19.2 g.
A2	♀	19.7
A3	♀	19.6
A4	♂	19.0
A5	♂	24.7
A6	♂	24.9

Group B 2 Hour.

B1	♀	19.2 g
B2	♀	20.9
B3	♀	18.9
B4	♂	23.6
B5	♂	19.6
B6	♂	21.6

Group C 4 Hour

C1	♀	16.7 (had large tongue)
C2	♀	20.3
C3	♀	20.3
C4	♂	22.9
C5	♂	24.0
C6	♂	23.9

CETP-Tg

Author's Signature <i>Murray Kekue</i>	Date 12-2-96	Read and Understood By <i>Robinson</i>	Date 1/27/99
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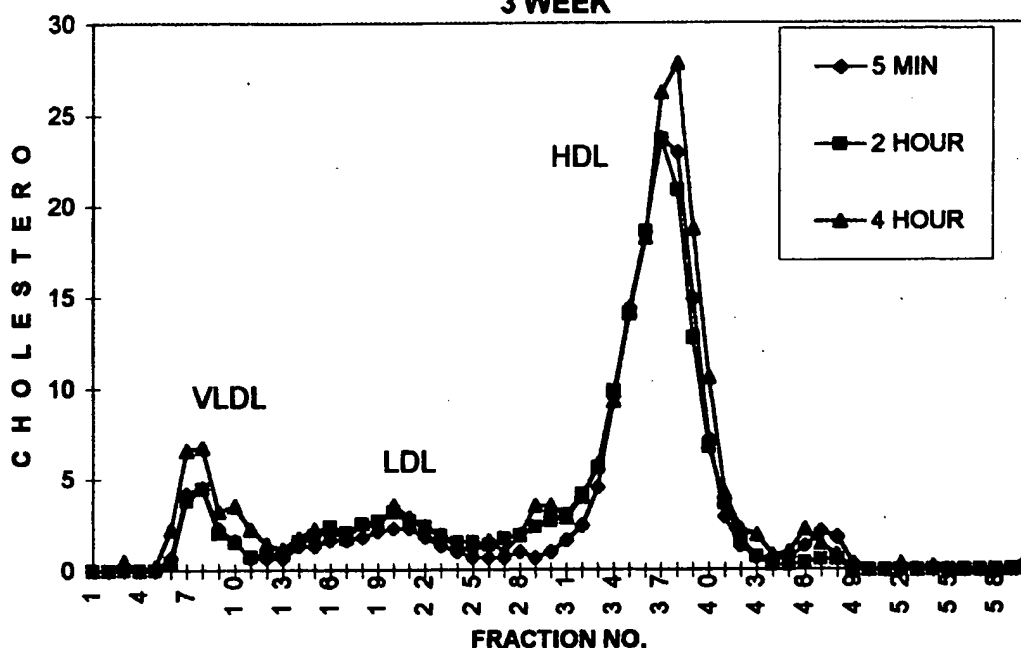
Project Number 565711	Subject cont.	Book Number GDS - 5748
SEARLE		Page 155

Pool 200 μ each / group. (Grp ~~B~~ #6 only 100 μ ,
 grp A #2 only 100 μ)
 filter
 apply 500 μ onto Superose 6 x 2 7.21.7

method 9 Run 5

FPLC SUMMARY TG5

3 WEEK



FPLC PROFILE				
TG5 3 WEEK				
	GROUP A	GROUP B	GROUP C	
	CETP-TG	CETP-TG	CETP-TG	
	5 MIN	2 HOUR	4 HOUR	
VLDL fx 6-13	13.4	12.3	22.5	
%	8	7	10	
LDL fx 14-29	22.3	33.7	34.2	
%	13	19	16	
HDL fx 30-47	130.5	129.7	155.2	
%	77	73	70.8	
TOTAL ug CHOL	170.3	178.8	219.3	

Author's Signature Dwight K. Kuo	Date 12-9-96	Read and Understood By [Signature]	Date 1/27/98
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199

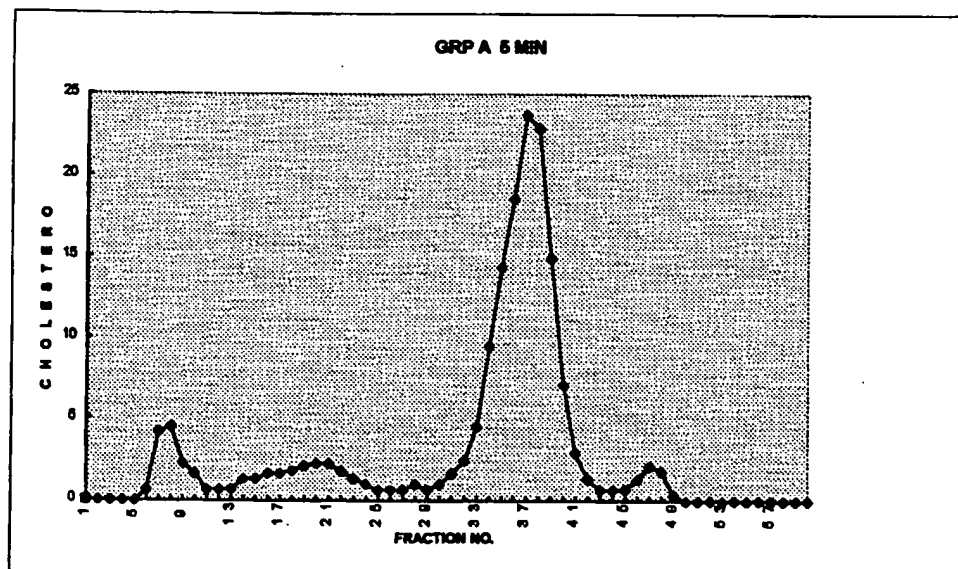
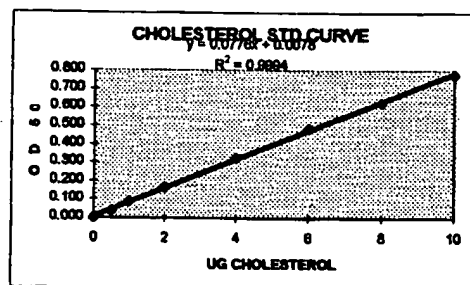
Book Number GDS - 5748	Subject T&S	Project Number 565711
Page 156	cont.	SEARLE

1	2	3	4	5	6	7	8	9	10	11	12
0.001	0.001	0.001	0.022	0.018	0.012	0.036	0.028	0.010	0.001	0.001	0.001
0.037	0.041	0.001	0.018	0.019	0.012	0.067	0.018	0.005	0.003	0.003	0.003
0.088	0.088	0.003	0.012	0.021	0.012	0.097	0.012	0.006	0.003	0.003	0.003
0.168	0.171	0.001	0.012	0.022	0.014	0.123	0.012	0.003	0.003	0.003	0.003
0.322	0.328	0.003	0.012	0.022	0.012	0.155	0.012	0.004	0.004	0.010	0.003
0.481	0.484	0.012	0.016	0.019	0.014	0.150	0.016	0.004	0.005	0.003	0.004
0.633	0.624	0.034	0.016	0.016	0.018	0.100	0.021	0.003	0.004	0.003	0.008
0.779	0.775	0.036	0.018	0.014	0.023	0.052	0.019	0.003	0.003	0.004	0.005

READ DATE:
12/2/96
ASSAY NAME:
GRP A 5 MIN
TG5 FPLC-A

CHOLESTEROL ASSAY

ug	STD	OD 1	OD 2	MEAN OD	SD OD	m	b					CALC STD
0	0.001	0.001	0.001	0.001	0.000							-0.088
0.5	0.037	0.041	0.039	0.039	0.003	0.0778	0.0078	#N/A	#N/A	#N/A	#N/A	0.402
1	0.088	0.088	0.088	0.088	0.000	0.0008	0.0040	#N/A	#N/A	#N/A	#N/A	1.034
2	0.168	0.171	0.169	0.169	0.004	0.9994	0.0074	#N/A	#N/A	#N/A	#N/A	2.072
4	0.322	0.328	0.325	0.325	0.004	#####	6.000	#N/A	#N/A	#N/A	#N/A	4.090
6	0.481	0.484	0.483	0.483	0.002	0.585	0.000	#N/A	#N/A	#N/A	#N/A	6.121
8	0.633	0.616	0.625	0.612	0.012							7.951
10	0.779	0.775	0.777	0.777	0.003							9.918



Author's Signature <i>Beverly Kipke</i>	Date 12-2-96	Read and Understood By <i>Bob</i>	Date 1/27/99
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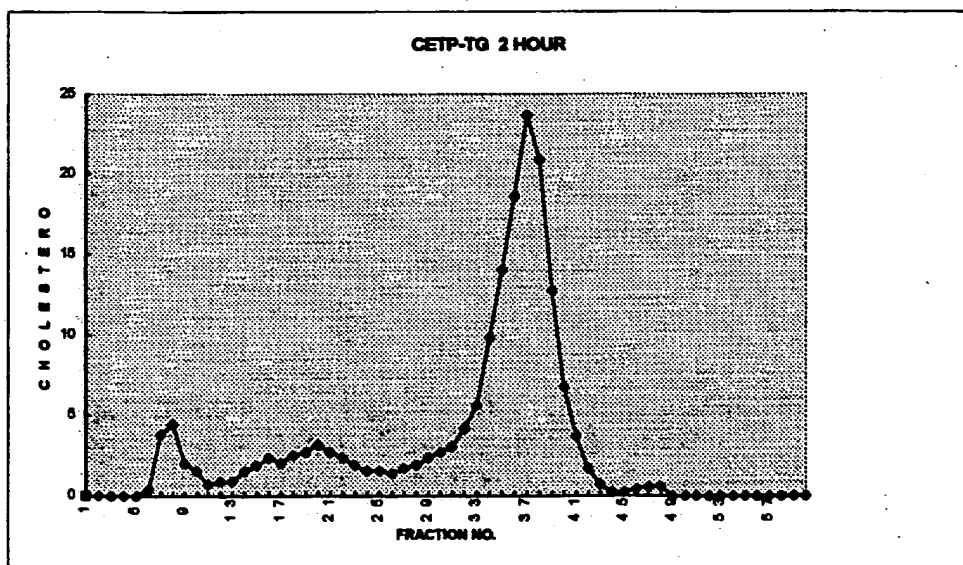
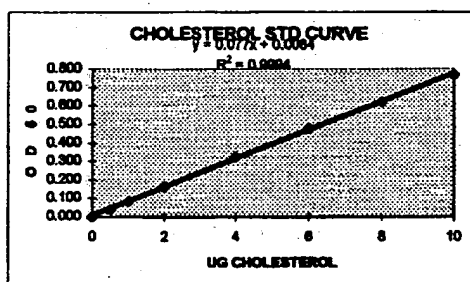
Author B

Project Number 565711	Subject TG-5	Book Number GDS - 5748
SEARLE	CHLT	Page 157

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.002	0.002	0.021	0.021	0.018	0.043	0.031	0.003	0.001	0.001	0.002
B	0.036	0.046	0.003	0.018	0.024	0.017	0.089	0.019	0.002	0.001	0.001	0.003
C	0.086	0.087	0.002	0.013	0.025	0.019	0.085	0.013	0.002	0.002	0.001	0.003
D	0.159	0.171	0.002	0.014	0.028	0.020	0.123	0.010	0.001	0.001	0.002	0.003
E	0.325	0.327	0.003	0.014	0.025	0.023	0.154	0.010	0.001	0.001	0.002	0.005
F	0.475	0.483	0.011	0.018	0.023	0.025	0.137	0.011	0.002	0.001	0.001	0.003
G	0.632	0.632	0.032	0.020	0.020	0.027	0.087	0.012	0.001	0.001	0.003	0.006
H	0.762	0.775	0.036	0.023	0.018	0.034	0.050	0.012	0.002	0.001	0.003	0.003

READ DATE:
12/2/88
ASSAY NAME:
CETP-TG 2 HOUR
TG5 FPLC-B

CHOLESTEROL ASSAY											
ug	STD	OD 1	OD 2	MEAN OD	SD OD						CALC STD
0	0.001	0.002	0.002	0.001	0.001	m	b				-0.090
0.5	0.036	0.046	0.041	0.007	0.007	0.0770	0.0064	#N/A	#N/A	#N/A	0.423
1	0.086	0.087	0.087	0.001	0.0008	0.0008	0.0041	#N/A	#N/A	#N/A	1.015
2	0.159	0.171	0.165	0.006	0.9994	0.0077	#N/A	#N/A	#N/A	#N/A	2.035
4	0.325	0.327	0.326	0.001	#####	6.000	#N/A	#N/A	#N/A	#N/A	4.127
6	0.475	0.483	0.479	0.006	0.578	0.000	#N/A	#N/A	#N/A	#N/A	6.115
8	0.632	0.616	0.624	0.011							7.999
10	0.762	0.775	0.769	0.006							9.877



Authority Signature Burley K. K.	Date 12-2-96	Read and Understood By R. Robison	Date 1/27/97
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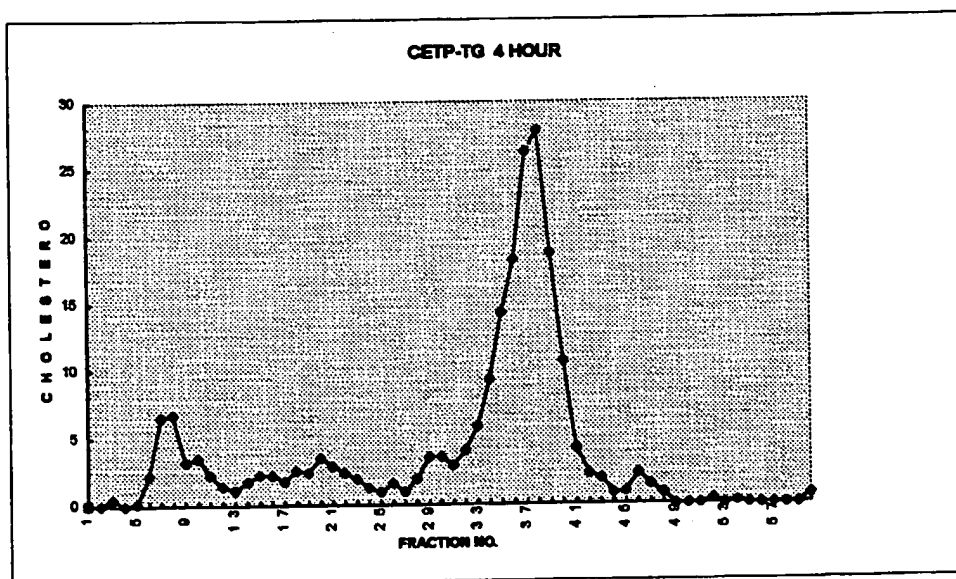
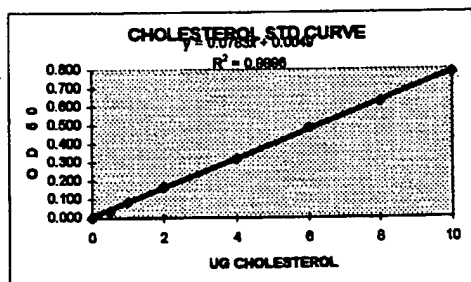
Book Number GDS - 5748	Subject T45	Project Number 505711
Page 158	Cont.	SEARLE

Project

1	2	3	4	5	6	7	8	9	10	11	12
0.001	0.001	-0.001	0.025	0.018	0.011	0.041	0.031	0.004	0.000	-0.002	-0.002
0.035	0.038	0.000	0.027	0.021	0.015	0.063	0.019	0.002	0.000	-0.001	0.000
0.088	0.087	0.008	0.019	0.020	0.011	0.084	0.017	0.003	0.003	0.005	0.002
0.168	0.168	0.000	0.014	0.027	0.017	0.119	0.010	0.007	0.009	0.007	0.002
0.320	0.317	0.006	0.012	0.023	0.027	0.169	0.011	0.004	0.006	-0.007	0.001
0.482	0.484	0.019	0.018	0.020	0.027	0.179	0.019	0.008	0.006	0.008	0.004
0.634	0.632	0.046	0.019	0.017	0.023	0.122	0.014	0.003	0.002	0.007	0.003
0.789	0.782	0.047	0.019	0.013	0.030	0.071	0.010	0.001	0.000	0.003	0.012

READ DATE:
12/2/88
ASSAY NAME:
CETP-TG 4 HOUR
TG5 FLC-C

CHOLESTEROL ASSAY											
ug	OD 1	OD 2	MEAN	SD							
STD	OD	OD	OD	OD	m	b	#N/A	#N/A	#N/A	#N/A	CALC
0	0.001	0.001	0.001	0.000							-0.049
0.5	0.035	0.038	0.037	0.002	0.0783	0.0049	#N/A	#N/A	#N/A	#N/A	0.404
1	0.088	0.087	0.087	0.001	0.0006	0.0034	#N/A	#N/A	#N/A	#N/A	1.043
2	0.168	0.168	0.168	0.000	0.9998	0.0063	#N/A	#N/A	#N/A	#N/A	2.085
4	0.320	0.317	0.319	0.002	#####	6.000	#N/A	#N/A	#N/A	#N/A	4.008
6	0.482	0.484	0.483	0.001	0.585	0.000	#N/A	#N/A	#N/A	#N/A	6.110
8	0.634	0.616	0.625	0.013							7.924
10	0.789	0.782	0.786	0.005							9.975

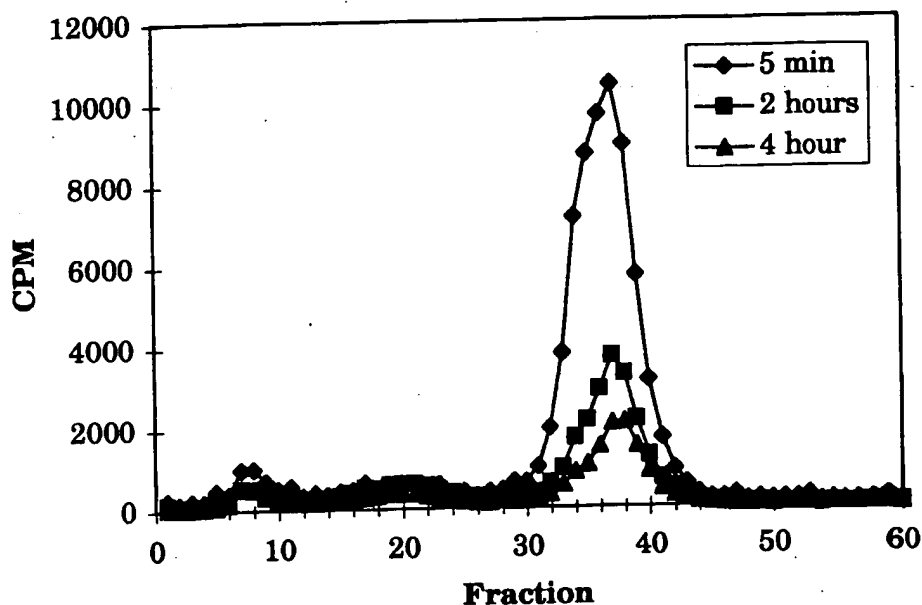


Author's Signature <i>Barbara K. Kline</i>	Date 12-2-96	Read and Understood By <i>[Signature]</i>	Date 1/27/97
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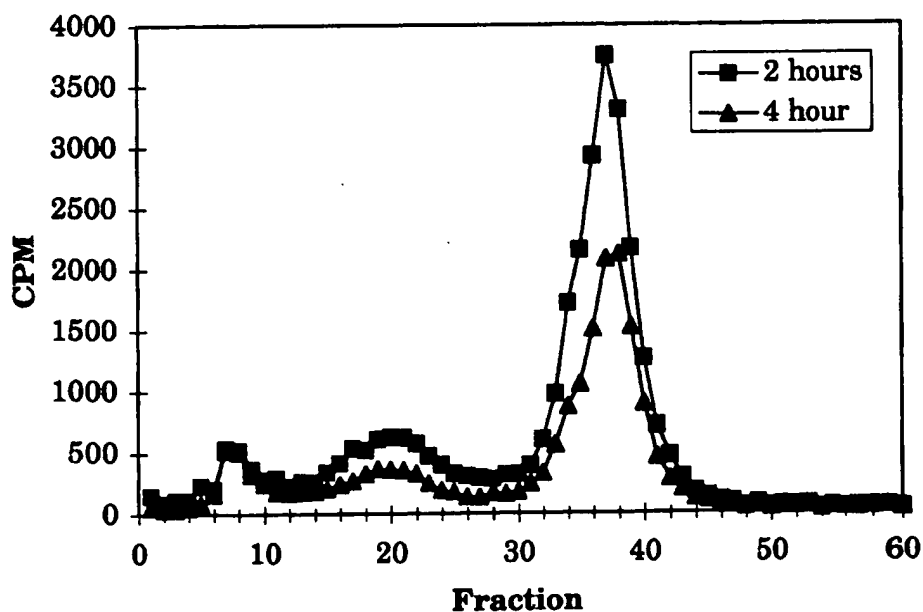
Author <i>[Signature]</i>

Project Number 505711	Subject TG 5, cont	Book Number GDS - 5748
SEARLE		Page 159

TG 5 CETP Activity in vivo



TG 5 CETP Activity in vivo



Author's Signature <i>Bruce Kubic</i>	Date 12-9-96	Read and Understood By <i>[Signature]</i>	Date 1/27/97
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Book Number GDS - 5748	Subject TB5, cont	Project Number 505711
Page 160		SEARLE

Project No.
S

	5 min	2 hour	4 hour
VLDL	5445	2904	2362
% of total	7	9	12
LDL	7992	6860	3850
% of total	10	21	20
HDL	65561	21545	12663
% of total	80	66	64
Total	81775	32471	19668
% transfered	16.43	30.07	31.58

12

F.

G

G1

Author's Signature <i>Beverly Kehue</i>	Date 12-9-96	Read and Understood By <i>[Signature]</i>	Date 1/27/99
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Author's 12

Project Number 505711	Subject TG6	Book Number GDS-5748
SEARLE	3 week diet	Page 161

12-5-96

3 groups, 6 animals each. All CETP-TG on 1% cholesterol diet for 3 weeks.
Inject 100 μ l HDL 151 retro M6. sinus (use CO₂)
3 time points: 5 min, 1 hour, 2 hour.
Rento luro IP for cardiac sticks. EDTA micro-tainer tubes.

F5 CETP-Tg # 248, 249, 250, 283, 285, 286, 287,
~~287~~ 288, 289, 296, 297, 303, 304, 305,
~~306~~ 307, 308, 309

Group A 5 min (actually 10 min.)

A1 ♀	23.6 g
A2 ♀	23.4
A3 ♀	23.9
A4 ♂	24.5
A5 ♂	26.4
A6 ♂	22.0

p.95 HDL 151

BK 12-10-96

HDL 151 = 16 μ Ci/ml

$.016 \mu\text{Ci/ml} \times 50 \mu\text{l} = 0.8 \mu\text{Ci}$

For disposal $< .05 \mu\text{Ci/g}$
20g mouse = 1 μCi

Dilute HDL 151 to 10 $\mu\text{Ci/ml}$
Inj. 100 μl

Group B 1 Hour

B1 ♀	19.7
B2 ♀	19.8
B3 ♀	18.8
B4 ♂	24.0
B5 ♂	25.2
B6 ♂	24.9

Group C 2 Hour

C1 ♀	19.2
C2 ♀	19.0
C3 ♀	22.9
C4 ♂	23.8
C5 ♂	25.8
C6 ♂	24.5

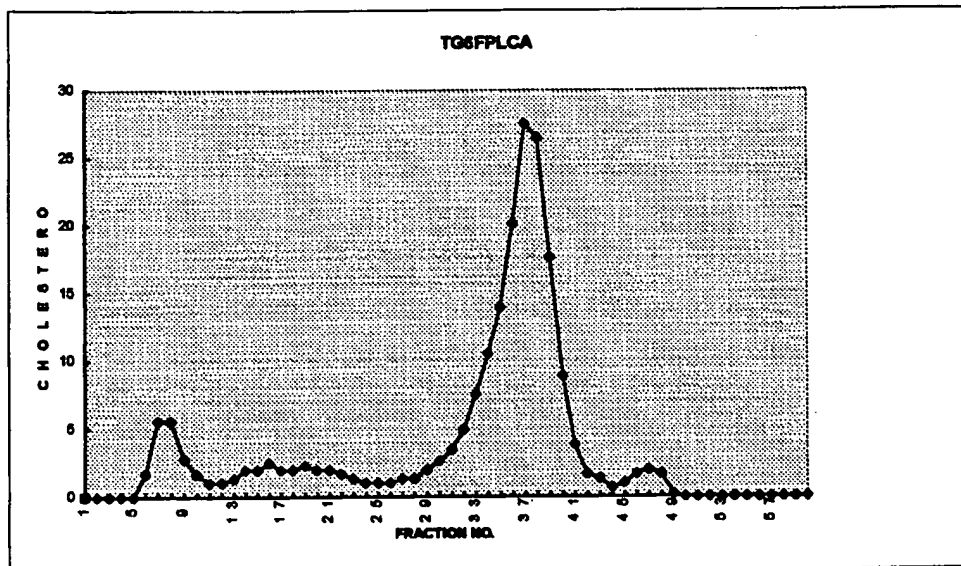
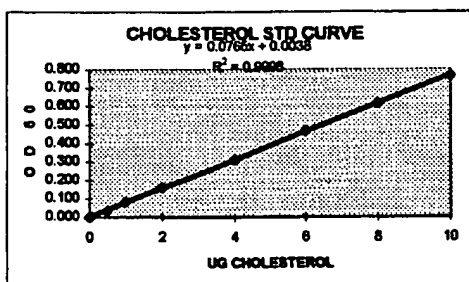
Author's Signature <i>Beverly Keene</i>	Date 12-9-96	Read and Understood By <i>AC (R) H. H. H.</i>	Date 1/27/97
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Book Number GDS - 5748	Subject TG 6	Project Number 505711
Page 162		SEARLE

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.001	0.001	0.021	0.016	0.010	0.050	0.027	0.005	0.001	0.001	-0.013
B	0.034	0.038	0.001	0.014	0.016	0.010	0.068	0.014	0.003	0.003	0.001	-0.012
C	0.083	0.080	0.003	0.010	0.018	0.012	0.089	0.012	0.001	0.001	0.001	-0.013
D	0.157	0.165	0.001	0.010	0.018	0.012	0.127	0.008	0.001	0.001	0.001	-0.012
E	0.306	0.323	0.003	0.012	0.016	0.016	0.172	0.010	0.001	0.001	0.001	-0.012
F	0.463	0.470	0.014	0.016	0.014	0.020	0.168	0.014	0.003	0.001	0.001	-0.012
G	0.615	0.621	0.038	0.016	0.012	0.025	0.112	0.016	0.003	0.001	0.003	-0.012
H	0.757	0.773	0.038	0.019	0.010	0.034	0.058	0.014	0.003	0.001	0.003	-0.012

READ DATE:
12/8/96
ASSAY NAME:
TG6FPLCA

CHOLESTEROL ASSAY											
ug	OD 1	OD 2	MEAN	SD							CALC
STD	OD 1	OD 2	OD	OD							STD
0	0.001	0.001	0.001	0.000	m	b					-0.036
0.5	0.034	0.038	0.036	0.003	0.0765	0.0038	#N/A	#N/A	#N/A	#N/A	0.421
1	0.083	0.080	0.082	0.002	0.0004	0.0023	#N/A	#N/A	#N/A	#N/A	1.016
2	0.157	0.165	0.161	0.006	0.8998	0.0044	#N/A	#N/A	#N/A	#N/A	2.054
4	0.306	0.323	0.315	0.012	#####	6.000	#N/A	#N/A	#N/A	#N/A	4.060
6	0.463	0.470	0.467	0.005	0.570	0.000	#N/A	#N/A	#N/A	#N/A	6.046
8	0.615	0.616	0.616	0.001							7.993
10	0.757	0.773	0.765	0.011							9.846



Author's Signature <i>Burney Kikie</i>	Date 12-9-96	Read and Understood By <i>Bob Robinson</i>	Date 1/27/89
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Project
Author

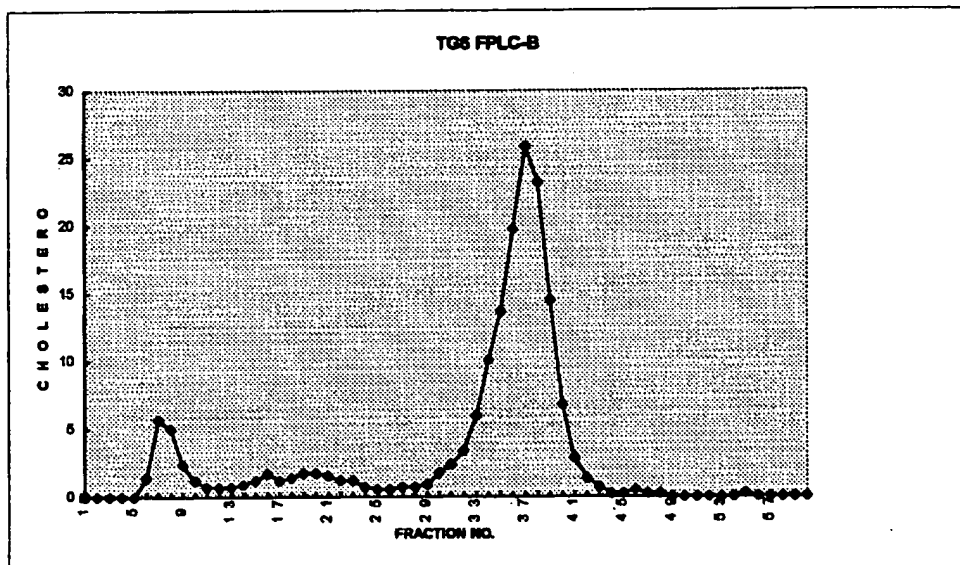
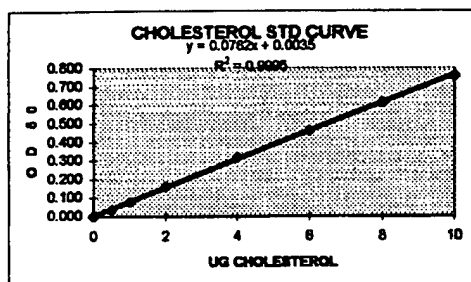
Project Number 565711	Subject TG 6	Book Number GDS - 5748
SEARLE		Page 163

1	2	3	4	5	6	7	8	9	10	11	12
0.001	0.000	-0.001	0.018	0.011	0.007	0.040	0.021	0.000	-0.002	-0.014	-0.013
0.033	0.036	-0.001	0.011	0.012	0.007	0.065	0.012	0.002	-0.002	-0.014	-0.014
0.082	0.074	-0.001	0.008	0.014	0.008	0.087	0.008	0.001	-0.002	-0.014	-0.015
0.159	0.164	0.000	0.008	0.014	0.008	0.124	0.005	-0.001	-0.002	-0.014	-0.015
0.315	0.324	0.001	0.008	0.013	0.008	0.161	0.005	-0.001	-0.002	-0.014	-0.014
0.457	0.464	0.012	0.009	0.011	0.014	0.145	0.007	0.003	-0.002	-0.014	-0.014
0.614	0.606	0.038	0.011	0.011	0.018	0.092	0.005	0.005	-0.001	-0.012	-0.013
0.762	0.757	0.034	0.014	0.008	0.024	0.045	0.005	0.000	-0.002	-0.010	-0.008

READ DATE:
12/9/98
ASSAY NAME:
TG6 FPLC-B

CHOLESTEROL ASSAY

ug	STD	OD 1	OD 2	MEAN	SD	OD	OD	m	b	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	CALC
0	0.001	0.000	0.001	0.001	0.001											-0.039
0.5	0.033	0.036	0.035	0.002	0.0762	0.0035	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	0.407
1	0.082	0.074	0.078	0.006	0.0007	0.0035	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	0.977
2	0.159	0.164	0.162	0.004	0.9995	0.0088	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	2.073
4	0.315	0.324	0.320	0.006	6.000	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	4.146
6	0.457	0.464	0.461	0.005	0.565	0.000	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	#N/A	5.996
8	0.614	0.616	0.615	0.001												8.023
10	0.762	0.757	0.760	0.004												9.919



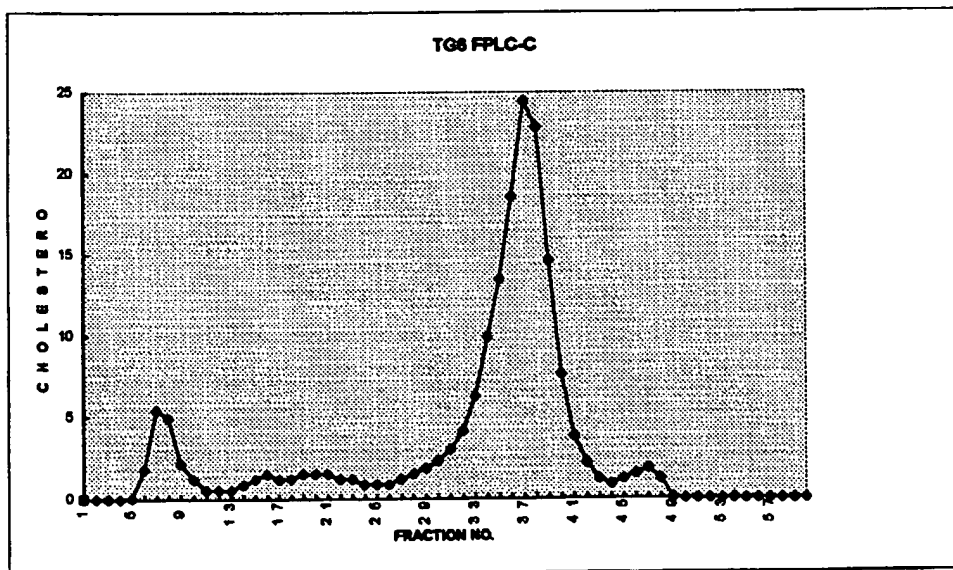
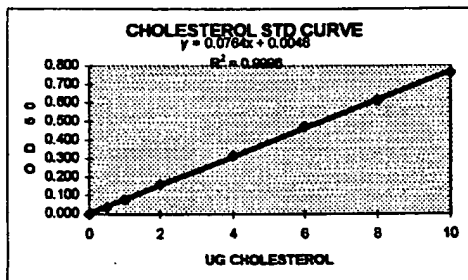
Author's Signature <i>Beverly Kake</i>	Date 12-9-96	Read and Understood By <i>Robson</i>	Date 1/27/99
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Book Number GDS - 5748	Subject TG 6 FPLC Raw data	Project Number 565711 SEARLE
Page 164		

Project #
S

	1	2	3	4	5	6	7	8	9	10	11	12	READ DATE:
A	0.001	0.001	0.001	0.018	0.012	0.010	0.043	0.028	0.005	0.001	0.001	0.001	12/8/98
B	0.034	0.039	0.001	0.012	0.012	0.010	0.065	0.018	0.001	0.001	0.001	0.001	ASSAY NAME:
C	0.077	0.084	0.001	0.008	0.014	0.012	0.087	0.012	0.001	0.001	0.001	0.001	TG8 FPLC-C
D	0.161	0.160	0.001	0.008	0.014	0.014	0.118	0.010	0.001	0.001	0.005	0.003	
E	0.313	0.322	0.005	0.008	0.014	0.016	0.154	0.012	0.001	0.001	0.001	0.003	
F	0.471	0.472	0.016	0.010	0.012	0.019	0.144	0.014	0.001	0.001	0.001	0.003	
G	0.610	0.612	0.038	0.012	0.012	0.023	0.094	0.016	0.001	0.001	0.001	0.003	
H	0.746	0.778	0.035	0.014	0.010	0.030	0.051	0.012	0.001	-0.001	0.001	0.003	

CHOLESTEROL ASSAY											
ug	OD 1	OD 2	MEAN	SD							CALC
STD	OD 1	OD 2	OD	OD	m	b					STD
0	0.001	0.001	0.001	0.000			#N/A	#N/A	#N/A	#N/A	-0.047
0.5	0.034	0.039	0.037	0.004	0.0764	0.0046	#N/A	#N/A	#N/A	#N/A	0.418
1	0.077	0.084	0.081	0.005	0.0006	0.0034	#N/A	#N/A	#N/A	#N/A	0.994
2	0.161	0.160	0.161	0.001	0.9996	0.0063	#N/A	#N/A	#N/A	#N/A	2.042
4	0.313	0.322	0.318	0.006	#####	6.000	#N/A	#N/A	#N/A	#N/A	4.097
6	0.471	0.472	0.472	0.001	0.567	0.000	#N/A	#N/A	#N/A	#N/A	6.113
8	0.610	0.616	0.613	0.004							7.966
10	0.746	0.778	0.762	0.023							9.917



Author's Signature <i>[Signature]</i>	Date 12-9-96	Read and Understood By <i>[Signature]</i>	Date 1/29/97
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Author's <i>[Signature]</i>

Author's Signature <i>Beverly Kepner</i>	Date 12-9-96	Read and Understood By <i>Mr. Ribner</i>	Date 1/27/97
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READ DATE:	12/9/96
ASSAY NAME:	TG6 TCHOL & HDL-CH

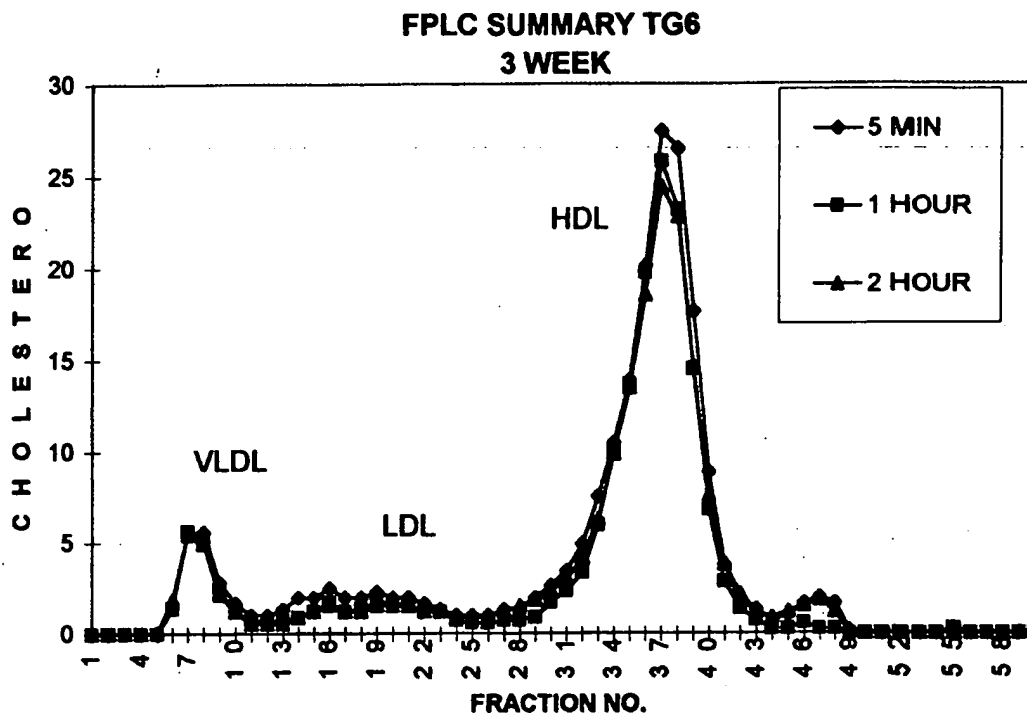
CHOLESTEROL STD CURVE

$y = 0.0712x + 0.0048$
 $R^2 = 0.9992$

ug CHOLESTEROL (x)	OD 50 (y)
0	0.0048
1	0.0760
2	0.1472
3	0.2184
4	0.2896
5	0.3608
6	0.4320
7	0.5032
8	0.5744
9	0.6456
10	0.7168

SAMPLE RESULTS (DUPLICATES)											
SAMP.	NO.	(ml)	OD 1	OD 2	MEAN	SD	CALC.	CALC.	DF	mg/dl	CHOL
			OD	OD	OD	ug	ug/ml				
	1	0.040	0.162	0.171	0.167	0.006	2.251	56.275	10.000	56.275	A1 TCHOL
	2	0.040	0.146	0.154	0.150	0.006	2.021	50.534	10.000	50.534	A2
	3	0.040	0.118	0.117	0.117	0.001	1.555	38.679	10.000	38.679	A3
	4	0.040	0.163	0.164	0.164	0.001	2.209	55.231	10.000	55.231	A4
	5	0.040	0.225	0.222	0.224	0.002	3.044	76.108	10.000	76.108	A5
	6	0.040	0.170	0.176	0.173	0.004	2.341	58.536	10.000	58.536	A6
	7	0.040	0.162	0.162	0.162	0.000	2.168	54.709	10.000	54.709	POOL A
	8	0.040	0.142	0.148	0.145	0.004	1.952	48.795	10.000	48.795	B1
	9	0.040	0.144	0.145	0.145	0.001	1.945	48.621	10.000	48.621	B2
	10	0.040	0.146	0.145	0.146	0.001	1.959	48.969	10.000	48.969	B3
	11	0.040	0.080	0.080	0.080	0.000	1.047	26.180	10.000	26.180	B4
	12	0.040	0.154	0.157	0.156	0.002	2.098	52.448	10.000	52.448	B5
	13	0.040	0.179	0.191	0.185	0.008	2.508	62.711	10.000	62.711	B6
	14	0.040	0.142	0.145	0.144	0.002	1.931	48.273	10.000	48.273	POOL B
	15	0.040	0.133	0.137	0.135	0.003	1.813	45.315	10.000	45.315	C1
	16	0.040	0.196	0.191	0.190	0.001	2.578	64.451	10.000	64.451	C2
	17	0.040	0.064	0.068	0.066	0.003	1.131	28.268	10.000	28.268	C3
	18	0.040	0.150	0.172	0.161	0.016	2.174	54.361	10.000	54.361	C4
	19	0.040	0.123	0.139	0.131	0.011	1.757	43.924	10.000	43.924	C5
	20	0.040	0.150	0.183	0.167	0.023	2.251	56.275	10.000	56.275	C6
	21	0.040	0.119	0.132	0.126	0.009	1.680	42.010	10.000	42.010	POOL C
	22	0.040	0.103	0.117	0.110	0.010	1.485	36.618	5.500	20.140	A1 HDL-CH
	23	0.040	0.096	0.103	0.100	0.005	1.319	32.985	5.500	18.130	A2
	24	0.040	0.087	0.098	0.093	0.008	1.221	30.529	5.500	16.791	A3
	25	0.040	0.265	0.268	0.276	0.013	3.768	94.197	5.500	51.809	A4
	26	0.040	0.352	0.343	0.348	0.008	4.770	119.247	5.500	65.586	A5
	27	0.040	0.196	0.183	0.191	0.011	2.585	64.625	5.500	35.544	A6
	28	0.040	0.254	0.247	0.251	0.005	3.420	85.499	5.500	47.025	POOL A
	29	0.040	0.164	0.149	0.157	0.011	2.112	52.796	5.500	29.038	B1
	30	0.040	0.164	0.175	0.180	0.008	2.432	60.798	5.500	33.438	B2
	31	0.040	0.171	0.164	0.168	0.005	2.285	56.823	5.500	31.142	B3
	32	0.040	0.117	0.111	0.114	0.004	1.520	38.009	5.500	20.905	B4
	33	0.040	0.231	0.243	0.237	0.008	3.232	80.803	5.500	44.441	B5
	34	0.040	0.247	0.278	0.263	0.022	3.587	89.874	5.500	49.321	B6
	35	0.040	0.197	0.198	0.198	0.001	2.682	67.080	5.500	36.883	POOL B
	36	0.040	0.106	0.105	0.107	0.002	1.316	36.400	5.500	19.470	C1
	37	0.040	0.245	0.245	0.245	0.000	3.343	83.586	5.500	45.972	C3
	38	0.040	0.193	0.194	0.194	0.001	2.627	65.658	5.500	36.118	C5
	39	0.040	0.250	0.243	0.247	0.005	3.384	84.108	5.500	48.259	C6
	40	0.040	0.236	0.231	0.234	0.004	3.183	79.585	5.500	43.772	POOL C

Book Number GDS - 5748	Subject TG6 Results	Project Number 565711
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FPLC PROFILE					
TG6 3 WEEK					
	GROUP A		GROUP B		GROUP C
	CETP-TG		CETP-TG		CETP-TG
	5 MIN		1 HOUR		2 HOUR
VLDL fx 5-13	17.4		15.7		15.8
%	8		9		9
LDL fx 14-29	27.6		18.2		20.4
%	13		11		11
HDL fx 30-47	155.9		133.6		140
%	76		79		78
TOTAL ug CHOL	206.1		170.2		179.2

Author's Signature <i>Barry Kehew</i>	Date <i>12-9-90</i>	Read and Understood By <i>Dr. Robinson</i>	Date <i>1/27/91</i>
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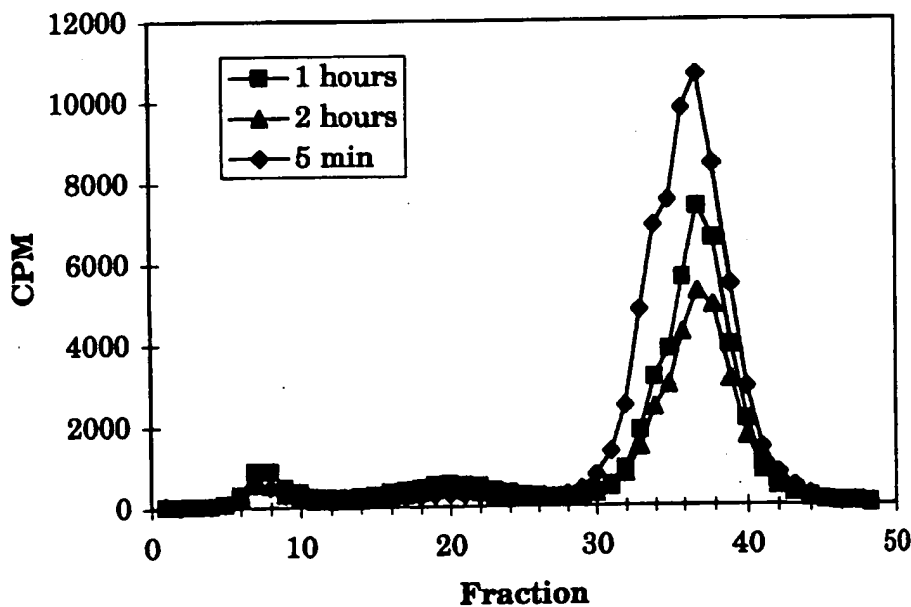
Author's
Dr. Robinson

Project Number 505711	Subject TG 6 Results	Book Number GDS - 5748
SEARLE		Page 167

TG6 3 WEEK	GROUP A CETP-TG 5 MIN	GROUP B CETP-TG 1 HOUR	GROUP C CETP-TG 2 HOUR
	56.3	48.8	45.3
	50.5	48.6	64.5
	38.9	49.0	28.3
	55.2	26.2	54.4
	76.1	52.4	43.9
	58.5	62.7	56.3
MEAN	55.9	48.0	48.8
STDEV	12.1	12.0	12.6
HDL-CH	20.1	29.0	
	18.1	33.4	
	16.8	31.1	19.5
	51.8	20.9	46.0
	65.6	44.4	36.1
	35.5	49.3	48.3
MEAN	34.7	34.7	37.0
STDEV	20.3	10.4	12.6

COUNTS

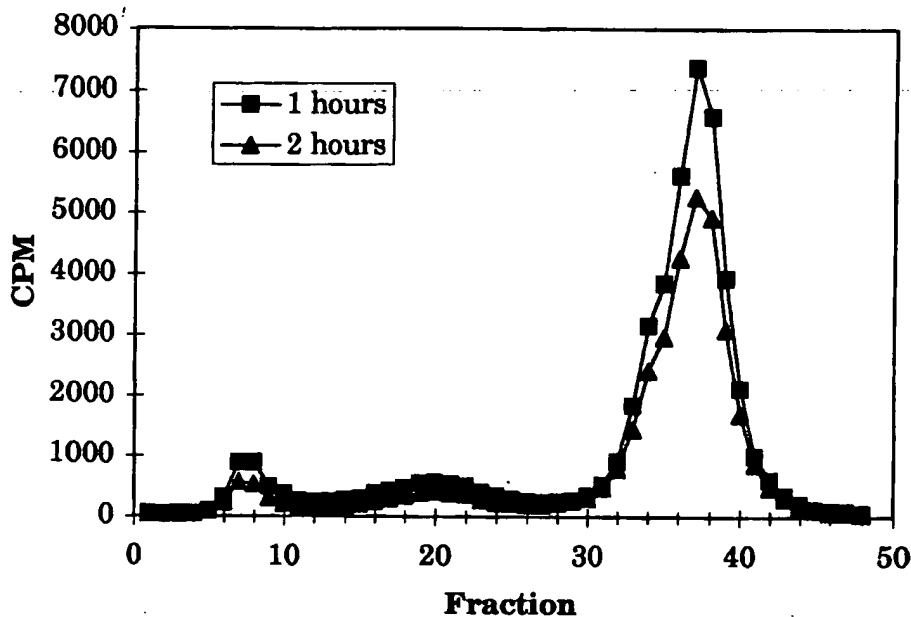
TG 6 CETP Activity in vivo



Author's Signature <i>Dwight K. Kober</i>	Date <i>12-9-96</i>	Read and Understood By <i>Ed R. Brown</i>	Date <i>1/27/97</i>
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Book Number GDS - 5748	Subject TG 6 Results	Project Number 565711
Page 168		SEARLE

TG 6 CETP Activity in vivo



	5 min	1 hour	2 hour
VLDL	2356	3795	2423
% of total	3	8	7
LDL	4092	6082	4324
% of total	6	12	12
HDL	64371	38698	29705
% of total	91	79	81
Total	71077	48806	36672
% transfered	9.07	20.24	18.40

Author's Signature <i>Barry Kewee</i>	Date 12-9-96	Read and Understood By <i>[Signature]</i>	Date 1/2/99
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Project Number 565711	Subject Rabbit Lipo protein Z-axis	Book Number GDS - 5748
SEARLE		Page 169

12-4-96 50 μ l rab. prep + 5 μ l Super blue
Use 8 μ l / lane

Pre-Run 10 min 100 V.

Lane 1 sera
2 ↓
3 ↓
4 filtered sera
5 VLDL
6 LDL
7 HDL
8 ????
9 HDL - bottom 11/20
10 HDL - middle 11/21

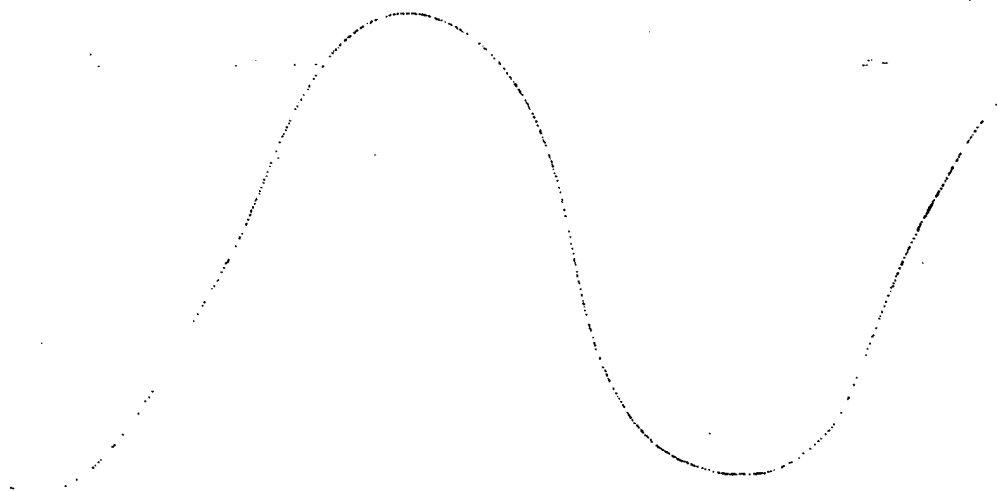
} comb was not set deep enough & sera spread.

lanes 7, 8, 9 : sample is higher in gel

Run 6 hours 100 V.

Stain overnight

Destain in Am with one change of destain.



Author's Signature D. W. K. Kene	Date 12-9-96	Read and Understood By G. R. Kene	Date 1/27/99
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Book Number GDS - 5748	Subject TG7	Project Number 565711
Page 170	3 week chol diet / 142 Hour	SEARLE

12-11-96

Projec
13

3 Groups : 6 animals each ~~1/27/99~~ CETP-Tg + Non-Tg

Group A 1 Hour CETP-Tg

A1	♀	19.2	> wt. taken before bleed
A2	♀	20.7	
A3	♀	18.5	
A4	♂	25.4	
A5	♂	23.9	
A6	♂	24.7	

Group B 2 Hour CETP-Tg

B1	♀	22.0
B2	♀	18.4
B3	♀	19.0
B4	♂	24.1
B5	♂	25.2
B6	♂	Died after 3H-HDL inj.

Group C 2 Hour Non-Tg Lot# 9960902

C1	♀	27.2
C2	♀	29.8
C3	♀	30.8
C4	♂	29.6
C5	♂	29.5
C6	♂	32.3

CETP-Tg # 310, 311, 321, 322-328, 294, 295

Author's Signature <i>Beverly Hoke</i>	Date 12-11-96	Read and Understood By <i>(Signature)</i>	Date 1/29/99
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Author's
✓

Project Number 505711	Subject Tg7	Book Number GDS - 5748
SEARLE		Page 171

12-11-96

All mice put on diet (Teklad 92181) (1% chol) on Nov. 20.

Mice were put down w/ CO₂ and 3H-HDL 151 (p. 161) 100 μ l inf orbital sinus.

After appropriate time mice were given pentobarb IP and cardiac sticks done.

Spin blood. Pool sera for FPLC

Grp A 150 μ l each filtered

500 μ l applied

Grp B 200 μ l #1, 2, 5 OETP-Tg 6

150 μ l #3

100 μ l #4

Grp C 200 μ l each A/Tg 6

Make 1:10 dilution of samples & pools for Tchol.

Count 200 μ l

Author's Signature Beverly Kieck	Date 12-12-96	Read and Understood By B. Kieck	Date 1/27/99
-------------------------------------	------------------	------------------------------------	-----------------

Book Number GDS - 5748	Subject TG 7	Project Number 505711
Page 172		SEARLE

Projec

1	2	3	4	5	6	7	8	9	10	11	12
0.001	0.001	0.175	0.175	0.131	0.134	0.180	0.158	-0.014	-0.014	-0.014	-0.013
0.033	0.038	0.155	0.155	0.124	0.130	0.189	0.184	-0.014	-0.013	-0.014	-0.013
0.089	0.085	0.170	0.169	0.194	0.185	0.221	0.218	-0.014	-0.013	-0.013	-0.013
0.159	0.169	0.084	0.085	0.158	0.168	0.181	0.177	-0.014	-0.013	-0.013	-0.013
0.318	0.324	0.214	0.213	0.166	0.170	0.001	0.002	-0.013	-0.013	-0.013	-0.011
0.471	0.479	0.182	0.185	0.153	0.163	0.002	0.001	-0.014	-0.013	-0.012	-0.013
0.614	0.630	0.163	0.167	0.164	0.165	0.002	0.002	-0.013	-0.013	-0.013	-0.013
0.758	0.787	0.205	0.205	0.167	0.175	0.000	0.000	-0.012	-0.013	-0.009	-0.013

READ DATE:

12/12/96

ASSAY NAME:

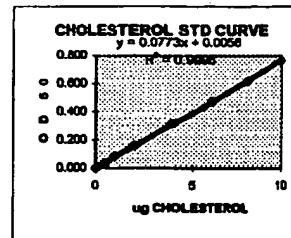
TG2-PEPPER

CHOLESTEROL ASSAY

ug STD	OD 1	OD 2	MEAN OD	SD OD	m	b						CALC STD
0	0.001	0.001	0.001	0.000								-0.058
0.5	0.033	0.038	0.038	0.004	0.0773	0.0058	#N/A	#N/A	#N/A	#N/A	#N/A	0.388
1	0.089	0.085	0.087	0.003	0.0006	0.0034	#N/A	#N/A	#N/A	#N/A	#N/A	1.054
2	0.159	0.169	0.184	0.007	0.9998	0.0063	#N/A	#N/A	#N/A	#N/A	#N/A	2.051
4	0.318	0.324	0.321	0.004	#####	6.000	#N/A	#N/A	#N/A	#N/A	#N/A	4.083
6	0.471	0.479	0.475	0.006	0.580	0.000	#N/A	#N/A	#N/A	#N/A	#N/A	6.077
8	0.614	0.630	0.622	0.011								7.979
10	0.758	0.787	0.773	0.021								9.927

SAMPLE RESULTS (DUPLICATES)

SAMP. NO.	(ml)	OD 1	OD 2	MEAN OD	SD OD	CALC. ug	CALC. ug/ml	DF	mg/dl CHOL	
1	0.040	0.175	0.173	0.174	0.001	2.180	54.508	10.000	54.508	A1
2	0.040	0.155	0.156	0.156	0.001	1.941	48.522	10.000	48.522	A2
3	0.040	0.170	0.169	0.170	0.001	2.122	53.052	10.000	53.052	A3
4	0.040	0.084	0.085	0.085	0.001	1.022	25.546	10.000	25.546	A4
5	0.040	0.214	0.213	0.214	0.001	2.692	67.291	10.000	67.291	A5
6	0.040	0.182	0.185	0.184	0.002	2.303	57.582	10.000	57.582	A6
7	0.040	0.163	0.167	0.165	0.003	2.084	51.598	10.000	51.598	POOL A
8	0.040	0.205	0.205	0.205	0.000	2.582	64.540	10.000	64.540	B1
9	0.040	0.131	0.134	0.133	0.002	1.843	41.079	10.000	41.079	B2
10	0.040	0.124	0.130	0.127	0.004	1.572	39.299	10.000	39.299	B3
11	0.040	0.184	0.185	0.180	0.006	2.381	59.524	10.000	59.524	B4
12	0.040	0.158	0.168	0.163	0.007	2.038	50.949	10.000	50.949	B5
13	0.040	0.166	0.170	0.168	0.003	2.103	52.567	10.000	52.567	POOL B
14	0.040	0.153	0.163	0.158	0.007	1.973	49.331	10.000	49.331	C1
15	0.040	0.164	0.166	0.165	0.001	2.057	51.434	10.000	51.434	C2
16	0.040	0.167	0.175	0.171	0.006	2.141	53.537	10.000	53.537	C3
17	0.040	0.180	0.156	0.158	0.003	1.973	49.331	10.000	49.331	C4
18	0.040	0.189	0.184	0.187	0.004	2.342	58.553	10.000	58.553	C5
19	0.040	0.221	0.218	0.219	0.004	2.758	68.909	10.000	68.909	C6
20	0.040	0.181	0.177	0.179	0.003	2.245	56.126	10.000	56.126	POOL C



TG7

3 WEEK

GROUP A
CETP-TG
1 HOUR

GROUP B
CETP-TG
2 HOUR

GROUP C
NON-TG
2 HOUR

T-CHOL

54.5

64.540

49.3

48.5

41.079

51.4

53.1

39.299

53.5

25.5

59.524

49.3

67.3

50.949

58.6

57.6

68.9

MEAN

51.1

51.1

55.2

STDEV

14.0

11.1

7.6

51.1
51.126

Author's Signature <i>Beverly Kiker</i>	Date 12-12-96	Read and Understood By <i>R. Robinson</i>	Date 1/29/97
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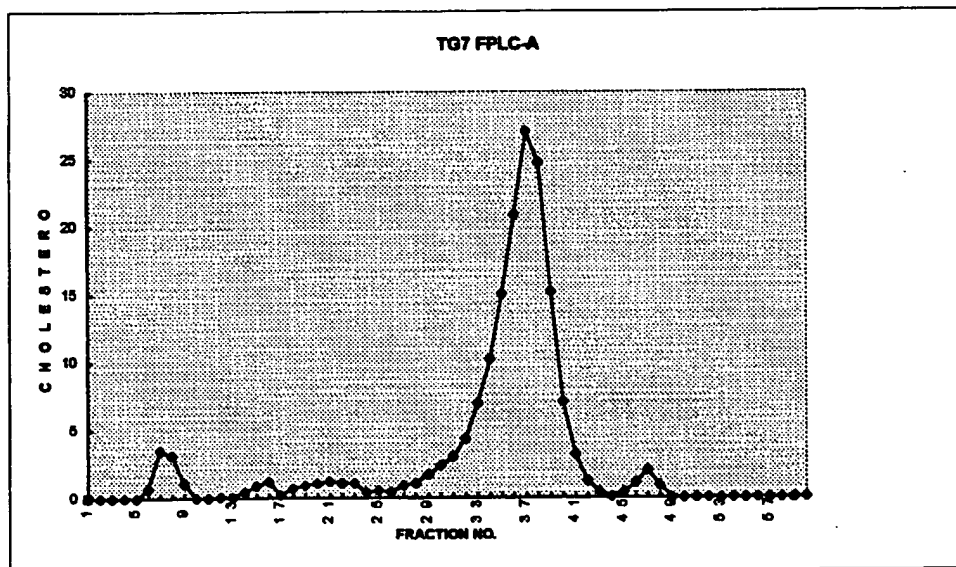
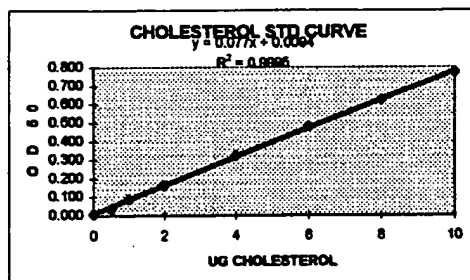
Author

Project Number 505711	Subject TG7	Book Number GDS - 5748
SEARLE		Page 173

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.002	0.020	0.000	0.016	0.011	0.013	0.052	0.029	0.003	0.001	-0.008	-0.010
B	0.038	0.040	0.002	0.009	0.014	0.012	0.072	0.017	0.002	0.002	-0.013	-0.013
C	0.088	0.089	0.002	0.008	0.015	0.015	0.102	0.012	0.003	0.001	0.011	-0.012
D	0.161	0.165	0.003	0.010	0.016	0.016	0.138	0.010	0.003	0.001	-0.012	-0.013
E	0.321	0.326	0.005	0.010	0.017	0.020	0.176	0.012	0.003	0.001	-0.010	-0.010
F	0.478	0.486	0.014	0.012	0.016	0.024	0.162	0.016	0.003	0.001	-0.008	-0.011
G	0.627	0.634	0.031	0.015	0.016	0.028	0.103	0.022	0.003	0.002	-0.012	-0.011
H	0.774	0.774	0.029	0.017	0.012	0.036	0.053	0.015	0.003	0.000	-0.008	-0.010

READ DATE:
12/12/96
ASSAY NAME:
TG7 FPLC-A

CHOLESTEROL ASSAY											
ug	STD	OD 1	OD 2	MEAN	SD						CALC
0	0.002	0.020	0.011	0.013		m	b				STD
0.5	0.036	0.040	0.038	0.003	0.0770	0.0094	#N/A	#N/A	#N/A	#N/A	0.021
1	0.086	0.089	0.088	0.002	0.0007	0.0037	#N/A	#N/A	#N/A	#N/A	0.371
2	0.161	0.165	0.163	0.003	0.9995	0.0070	#N/A	#N/A	#N/A	#N/A	1.014
4	0.321	0.326	0.324	0.004	#####	8.000	#N/A	#N/A	#N/A	#N/A	1.995
6	0.478	0.486	0.482	0.006	0.576	0.000	#N/A	#N/A	#N/A	#N/A	4.080
8	0.627	0.616	0.622	0.008							6.138
10	0.774	0.774	0.774	0.000							7.850
											9.931



Author's Signature <i>Beverly Kikes</i>	Date 12-12-96	Read and Understood By <i>G Robinson</i>	Date 1/27/99
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Book Number GDS - 5748	Subject TG7	Project Number 505711
Page 174		SEARLE

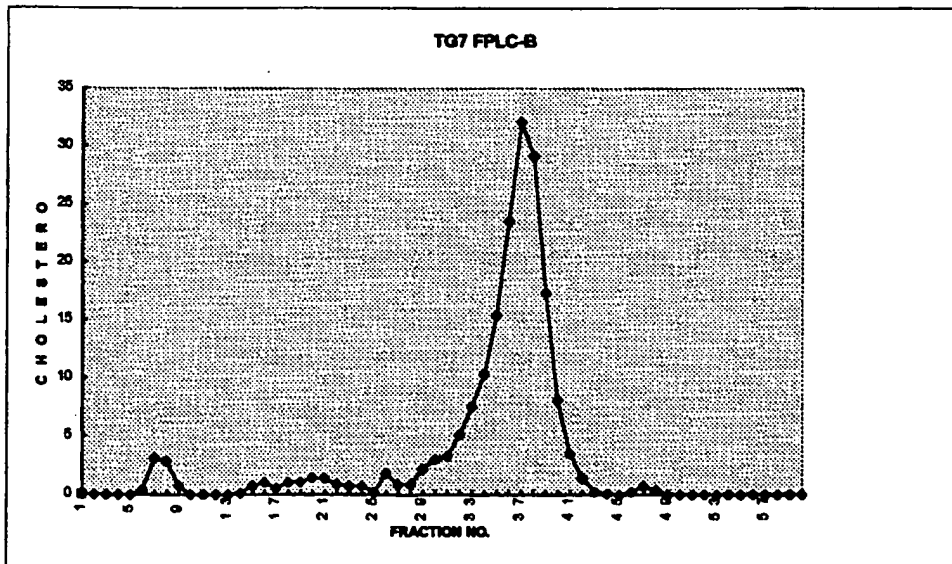
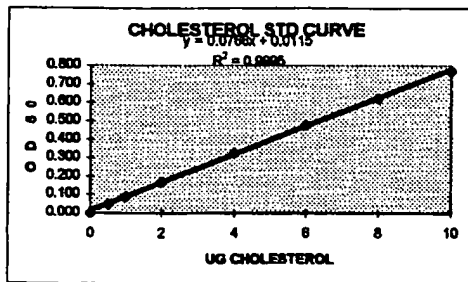
Prof

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.002	0.003	0.016	0.015	0.013	0.058	0.033	0.008	0.003	-0.010	-0.011
B	0.056	0.042	0.002	0.010	0.018	0.023	0.075	0.020	0.006	0.004	-0.010	-0.011
C	0.090	0.089	0.002	0.010	0.018	0.017	0.108	0.013	0.005	0.004	-0.010	-0.011
D	0.165	0.171	0.004	0.010	0.020	0.017	0.158	0.012	0.005	0.004	-0.010	-0.011
E	0.322	0.328	0.005	0.010	0.020	0.025	0.208	0.011	0.006	0.004	-0.009	-0.009
F	0.476	0.482	0.014	0.012	0.017	0.030	0.190	0.013	0.005	0.004	-0.009	-0.010
G	0.628	0.633	0.031	0.016	0.016	0.032	0.118	0.016	0.006	0.006	-0.009	-0.010
H	0.761	0.782	0.029	0.018	0.016	0.043	0.061	0.014	0.006	0.004	-0.008	-0.010

READ DATE:
12/12/88
ASSAY NAME:
TG7 FPLC-8

CHOLESTEROL ASSAY

ug	STD	OD 1	OD 2	MEAN	SD							CALC
0	0.001	0.002	0.002	0.001		m	b					-0.130
0.5	0.056	0.042	0.049	0.010	0.0768	0.0115	#N/A	#N/A	#N/A	#N/A	#N/A	0.490
1	0.090	0.089	0.090	0.001	0.0007	0.0036	#N/A	#N/A	#N/A	#N/A	#N/A	1.018
2	0.165	0.171	0.168	0.004	0.8995	0.0067	#N/A	#N/A	#N/A	#N/A	#N/A	2.043
4	0.322	0.328	0.325	0.004	#####	6.000	#N/A	#N/A	#N/A	#N/A	#N/A	4.092
6	0.476	0.482	0.479	0.004	0.571	0.000	#N/A	#N/A	#N/A	#N/A	#N/A	6.101
8	0.628	0.616	0.622	0.008								7.968
10	0.761	0.782	0.772	0.015								9.919



Author's Signature Beverly Kepke	Date 12-12-96	Read and Understood By [Signature]	Date 1/27/99
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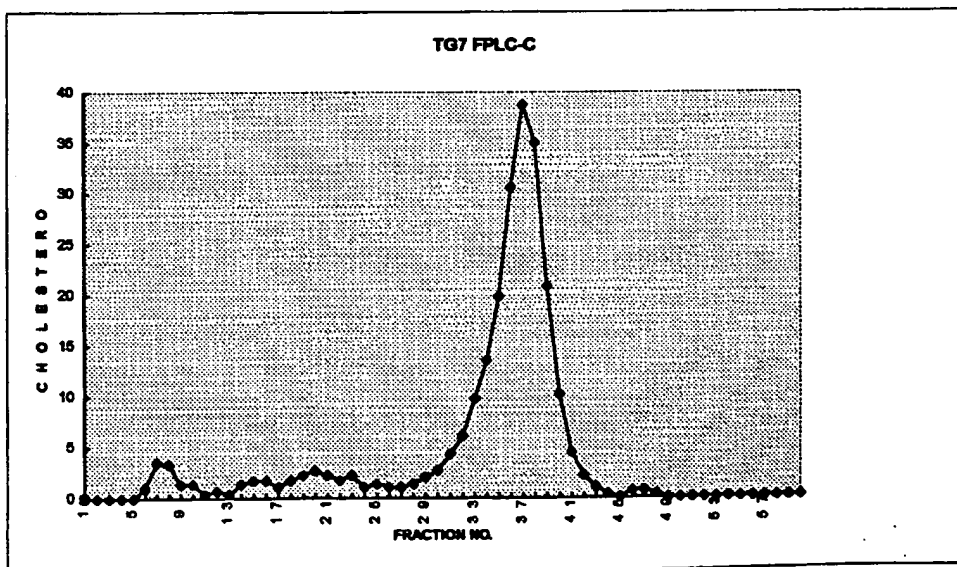
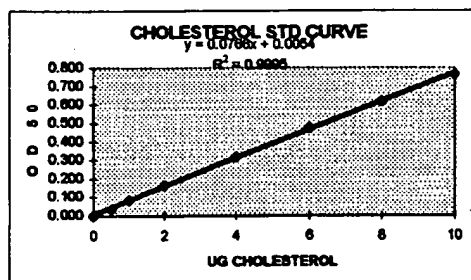
Auth

Project Number 505711	Subject TG7	Book Number GDS - 5748
SEARLE		Page 175

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.001	0.001	0.014	0.012	0.014	0.065	0.033	0.003	0.001	-0.014	-0.014
B	0.034	0.037	0.001	0.014	0.016	0.012	0.088	0.019	0.001	0.001	-0.014	-0.015
C	0.082	0.088	0.001	0.008	0.019	0.012	0.127	0.012	0.001	-0.001	-0.014	-0.014
D	0.160	0.167	0.001	0.010	0.022	0.014	0.183	0.008	0.001	0.001	-0.014	-0.014
E	0.312	0.319	0.003	0.008	0.019	0.018	0.243	0.006	0.001	-0.001	-0.014	-0.014
F	0.470	0.479	0.012	0.014	0.016	0.022	0.220	0.010	0.001	-0.001	-0.013	-0.014
G	0.616	0.624	0.027	0.016	0.019	0.032	0.133	0.010	0.001	-0.001	-0.013	-0.014
H	0.754	0.777	0.026	0.016	0.012	0.043	0.068	0.008	0.001	-0.001	-0.012	-0.014

READ DATE:
12/12/86
ASSAY NAME:
TG7 FPLC-C

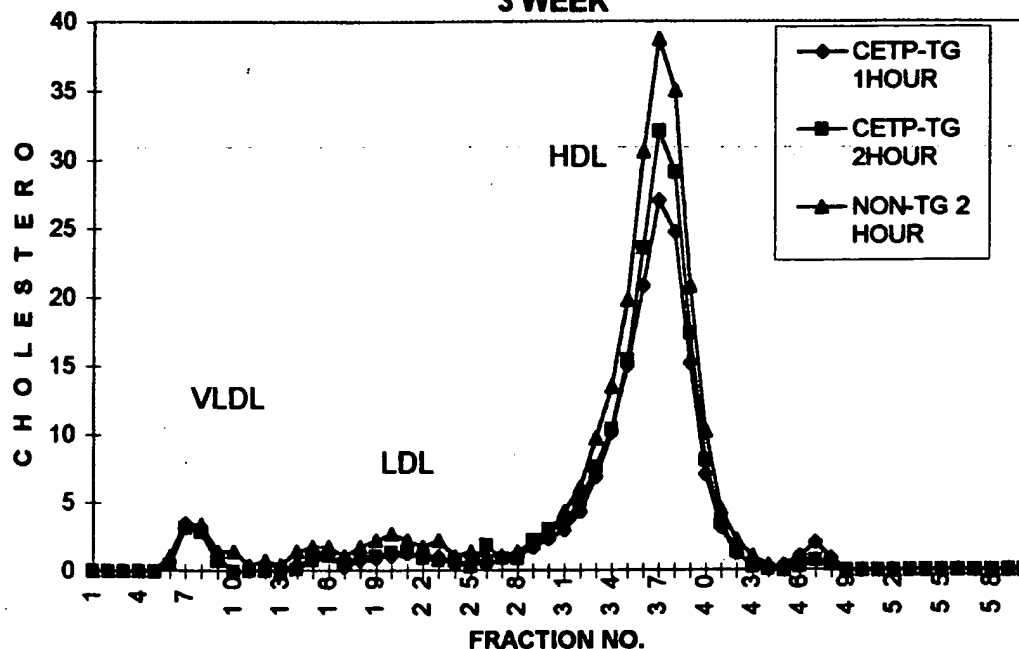
CHOLESTEROL ASSAY											
ug	STD	OD 1	OD 2	MEAN OD	SD OD	m	b				CALC STD
0	0	0.001	0.001	0.001	0.000						-0.057
0.5	0.034	0.037	0.037	0.036	0.002	0.0768	0.0054	#N/A	#N/A	#N/A	0.393
1	0.082	0.088	0.085	0.085	0.004	0.0007	0.0035	#N/A	#N/A	#N/A	1.039
2	0.160	0.167	0.164	0.005	0.005	0.9996	0.0066	#N/A	#N/A	#N/A	2.064
4	0.312	0.319	0.316	0.005	0.005	#####	6.000	#N/A	#N/A	#N/A	4.048
6	0.470	0.479	0.475	0.006	0.571	0.000	#N/A	#N/A	#N/A	#N/A	6.123
8	0.616	0.616	0.616	0.000							7.989
10	0.754	0.777	0.766	0.016							9.820



Author's Signature Beverly Kekic	Date 12-12-96	Read and Understood By [Signature]	Date 1/27/89
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Book Number GDS - 5748	Subject TG7	Project Number 505711
Page 176		SEARLE

**FPLC SUMMARY TG7
3 WEEK**



FPLC PROFILE				
TG7 3 WEEK				
	GROUP A	GROUP B	GROUP C	
	CETP-TG	CETP-TG	NON-TG	
	1 HOUR	2 HOUR	2 HOUR	
VLDL fx 5-13	8.5	7.2	10.8	
%	5	4	5	
LDL fx 14-29	14	15.9	27	
%	8	9	11	
HDL fx 30-47	145.2	161.3	201.6	
%	86	87	84	
TOTAL ug CHOL	168.8	184.9	241	

counts from FPLC - FPLC.058

Author's Signature <i>Beverly Kehue</i>	Date <i>12-12-96</i>	Read and Understood By <i>[Signature]</i>	Date <i>1/12/97</i>
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Author's

Project Number 505711 SEARLE	Subject T68 4 week	Book Number GDS - 5748 Page 177
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12-18-96

(Teklad 92181)

12 CETP-TG mice on 1% Chol. diet for 4 weeks
(since Nov. 20)

300, 301, 302, 312, 313, 314, 315, 316, 317,
318, 319, 320
all F5

6 NON-TG mice on 1% Chol. diet since Nov. 20

BD 6-1-96

lot 996 0902

Inj. 100 μ l 3H-HDL (p. 161) into sinus cavity, under
CO₂

Group A - CETP-Tg 1 Hour

Group B - CETP-Tg 2 Hour

Group C - Non-Tg 2 Hour - all died after inj. of HDL

Reason for death unknown - stress, age?

A1	♀	31.5	B1	♀	21.8
A2	♀	20.3	B2	♀	22.0
A3	♀	20.6 (Died)	B3	♀	31.3
A4	♂	28.0	B4	♂	23.9
A5	♂	24.4	B5	♂	24.3
A6	♂	27.9	B6	♂	26.2

Group A+B - 200 μ l each pooled (6/group, group A only)
FPLC
buffer, 500 μ l applied to FPLC

Author's Signature M. W. K. K. K.	Date 12-20-96	Read and Understood By M. W. K. K. K.	Date 1/27/97
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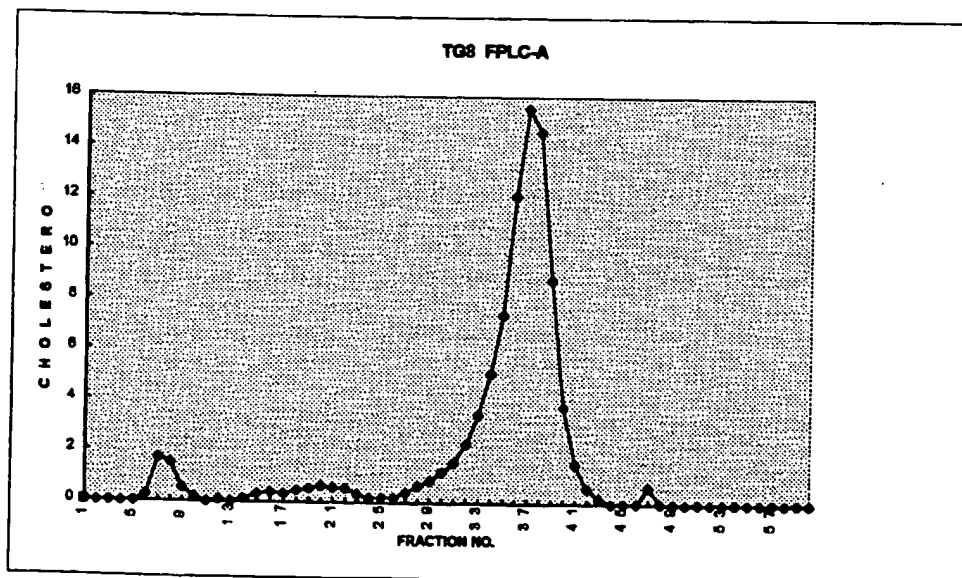
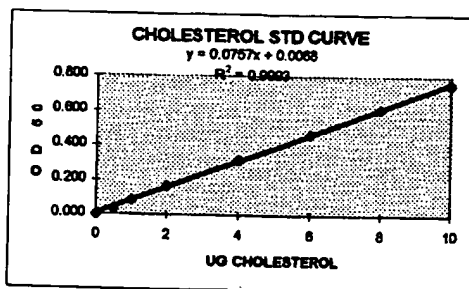
Book Number GDS - 5748	Subject TG 8	Project Number 505711
Page 178	FPLC - Chol	SEARLE

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.001	0.001	0.015	0.012	0.010	0.059	0.030	0.001	-0.001	-0.015	-0.015
B	0.031	0.038	-0.001	0.010	0.014	0.010	0.084	0.018	0.002	-0.001	-0.015	-0.015
C	0.083	0.087	0.000	0.007	0.015	0.013	0.119	0.010	0.000	0.000	-0.015	-0.014
D	0.157	0.172	-0.001	0.008	0.017	0.017	0.190	0.007	-0.001	-0.001	-0.014	-0.014
E	0.311	0.325	0.001	0.007	0.018	0.020	0.242	0.007	-0.001	-0.002	-0.014	-0.015
F	0.487	0.470	0.011	0.008	0.015	0.025	0.228	0.007	0.000	-0.001	-0.014	-0.013
G	0.609	0.618	0.033	0.012	0.012	0.031	0.140	0.017	0.000	-0.002	-0.015	-0.015
H	0.755	0.755	0.030	0.013	0.010	0.042	0.064	0.007	0.000	-0.002	-0.014	-0.014

READ DATE:
12/20/88
ASSAY NAME:
TG8 FPLC-A
CETP-TG
4 WK/1 HOUR

CHOLESTEROL ASSAY

ug	OD 1	OD 2	MEAN	SD								CALC
STD	OD	OD	OD	OD								STD
0	0.001	0.001	0.001	0.000								-0.076
0.5	0.031	0.038	0.035	0.005	m	b						0.368
1	0.083	0.087	0.085	0.003	0.0757	0.0068	#N/A	#N/A	#N/A	#N/A		1.033
2	0.157	0.172	0.165	0.011	0.0008	0.0043	#N/A	#N/A	#N/A	#N/A		2.083
4	0.311	0.325	0.318	0.010	0.9993	0.0080	#N/A	#N/A	#N/A	#N/A		4.111
6	0.487	0.470	0.469	0.002	#####	6.000	#N/A	#N/A	#N/A	#N/A		6.099
8	0.609	0.618	0.613	0.005	0.557	0.000	#N/A	#N/A	#N/A	#N/A		8.001
10	0.755	0.755	0.755	0.000								9.883



Author's Signature <i>Murray Kikic</i>	Date <i>12-30-96</i>	Read and Understood By <i>[Signature]</i>	Date <i>1/27/97</i>
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Author's <i>[Signature]</i>

Project Number 565711	Subject TC8	Book Number GDS-5748
SEARLE	FPLC-chole	Page 179

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.001	0.001	0.022	0.015	0.017	0.067	0.034	0.005	0.003	-0.012	-0.013
B	0.028	0.042	0.001	0.015	0.017	0.025	0.100	0.019	0.004	0.001	-0.012	-0.011
C	0.081	0.090	0.001	0.011	0.018	0.020	0.110	0.013	0.003	0.003	-0.012	-0.012
D	0.164	0.162	0.002	0.011	0.018	0.022	0.168	0.010	0.003	0.004	-0.011	-0.012
E	0.322	0.320	0.004	0.012	0.018	0.029	0.231	0.008	0.004	0.002	-0.011	-0.012
F	0.481	0.487	0.016	0.013	0.016	0.032	0.220	0.015	0.003	0.003	-0.011	-0.013
G	0.617	0.621	0.044	0.016	0.019	0.037	0.135	0.012	0.002	0.001	-0.012	-0.011
H	0.768	0.763	0.039	0.017	0.013	0.048	0.071	0.010	0.002	0.000	-0.012	-0.012

READ DATE:

12/20/86

ASSAY NAME:

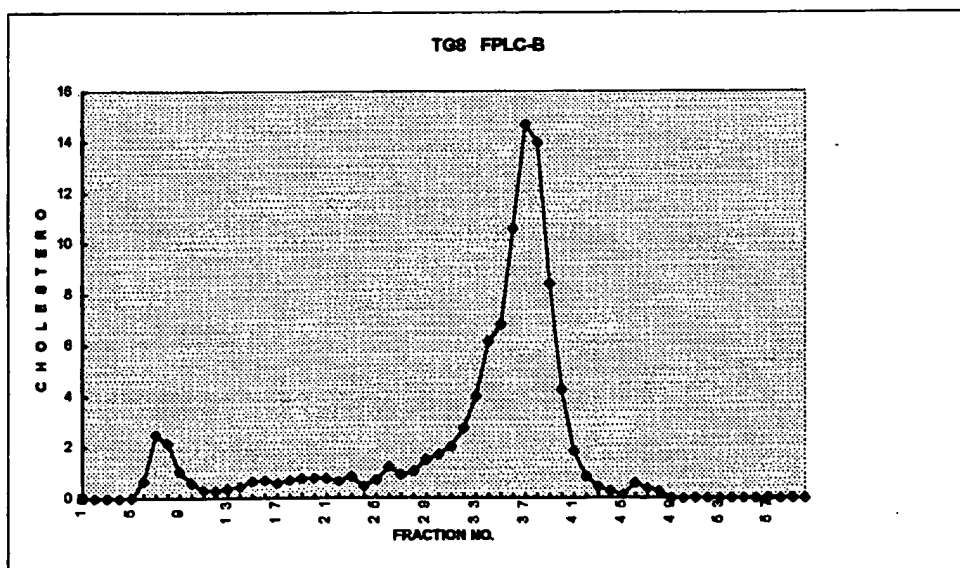
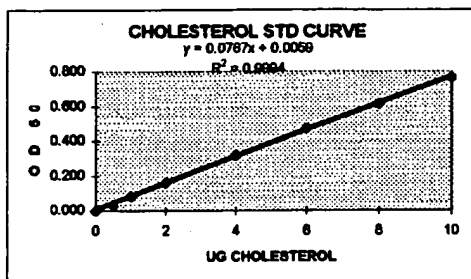
TG8 FPLC-B

CETP-TG

2 HOUR

CHOLESTEROL ASSAY

ug	OD 1	OD 2	MEAN	SD								CALC
STD			OD	OD								STD
0	0.001	0.001	0.001	0.000								-0.084
0.5	0.028	0.042	0.035	0.010	0.0787	0.0059	#N/A	#N/A	#N/A	#N/A	#N/A	0.380
1	0.081	0.090	0.086	0.006	0.0007	0.0039	#N/A	#N/A	#N/A	#N/A	#N/A	1.039
2	0.164	0.162	0.163	0.001	0.9994	0.0074	#N/A	#N/A	#N/A	#N/A	#N/A	2.050
4	0.322	0.320	0.321	0.001	#####	6.000	#N/A	#N/A	#N/A	#N/A	#N/A	4.111
6	0.481	0.487	0.474	0.018	0.571	0.000	#N/A	#N/A	#N/A	#N/A	#N/A	6.107
8	0.617	0.616	0.617	0.001								7.966
10	0.768	0.763	0.766	0.004								9.910



Author's Signature <i>Dwerry Keker</i>	Date 12-20-86	Read and Understood By <i>[Signature]</i>	Date 1/27/89
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Project

READ DATE:
12/20/98
ASSAY NAME:
TG8 TCHOL

CHOLESTEROL STD CURVE
 $y = 0.0756x + 0.0061$
 $R^2 = 0.9999$

The graph shows a linear relationship between absorbance (y-axis) and cholesterol concentration in μg (x-axis). The x-axis ranges from 0 to 10 μg with major ticks every 5 units. The y-axis ranges from 0.000 to 0.600 with major ticks every 0.200 units. A series of data points are plotted, showing a very strong linear correlation. A regression line is drawn through the points.

Cholesterol Concentration (μg)	Absorbance
0	0.0061
1	0.0817
2	0.1573
3	0.2329
4	0.3085
5	0.3841
6	0.4597
7	0.5353
8	0.6109
9	0.6865
10	0.7621

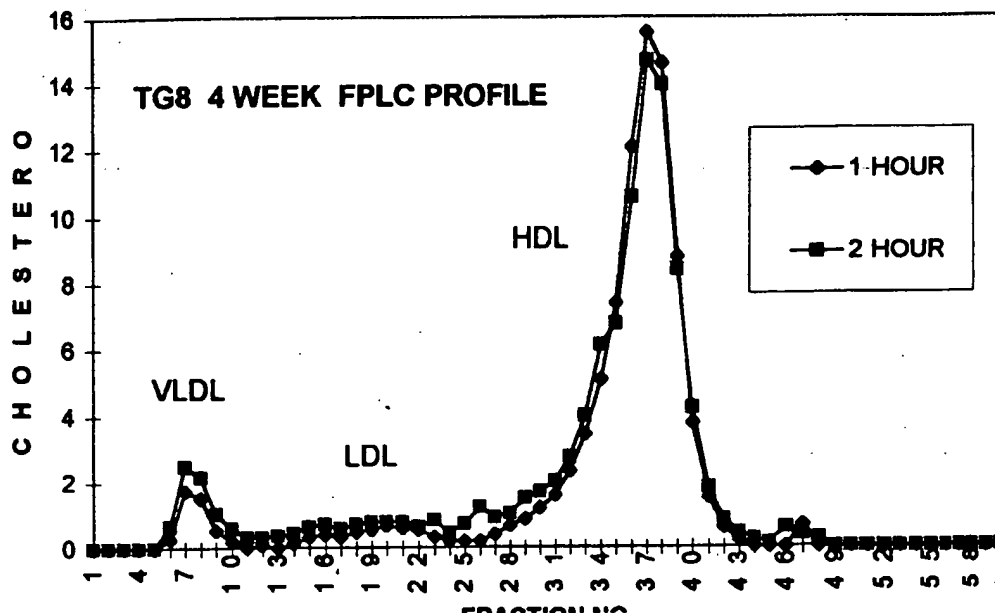
T 8
4 WEEK
ALL CETP-TG

	GROUP A 1 HOUR	GROUP B 2 HOUR
	61.8	64.3
	54.7	67.6
		54.5
	62.8	69.7
	61.0	
	68.6	79.8
MEAN	61.8	67.2
STDEV	5.0	9.2

Total chol are about the same between the 2 different time periods.

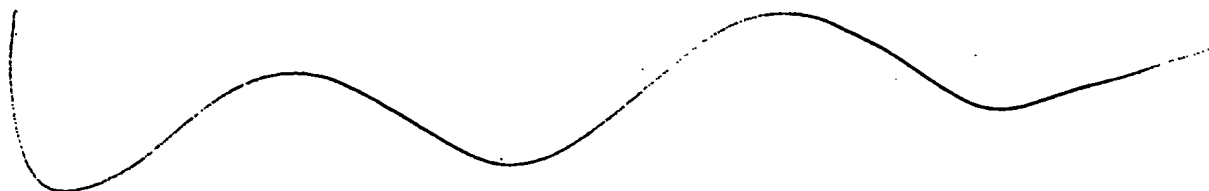
Author: 

Project Number 565711	Subject Cont,	Book Number GDS - 5748
SEARLE		Page 181



TG8			
4 WEEK			
ALL CETP-TG			
	GROUP A	GROUP B	
	CETP-TG	CETP-TG	
	1 HOUR	2 HOUR	
VLDL fx 5-13	4.3	7	
%	5	7	
LDL fx 14-29	7.1	13	
%	8	13	
HDL fx 30-47	79	79.9	
%	87	79	
TOTAL ug CHOL	90.5	101.1	

The cholesterol profile is about the same between the two different time points



Author's Signature <i>Beverly K. K...</i>	Date 12-20-96	Read and Understood By <i>[Signature]</i>	Date 1/27/99
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Book Number GDS - 5748	Subject TB 8 4 wk study	Project Number 565711
Page 182		SEARLE

TG8 CETP Activity/ 4 weeks on diet

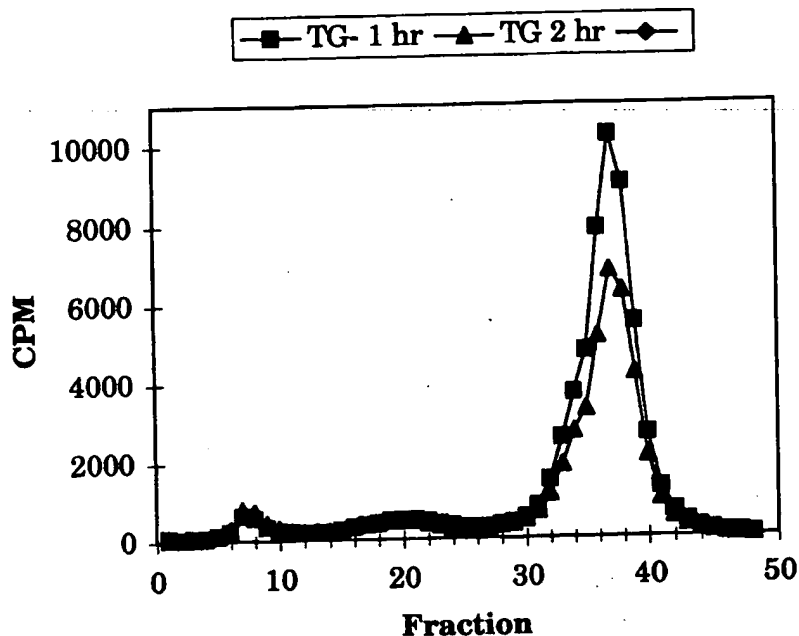
fmn #	TG- 1 hr CPM	TG 2 hr CPM
1	83.76	61.44
2	46.24	49.4
3	69.86	55.66
4	67.28	67.12
5	182.36	194.2
6	244.56	294.14
7	619.28	782.76
8	558.76	728.68
9	349.62	443.08
10	241.76	305.9
11	223.76	239.28
12	177.12	222.54
13	214.02 2761	240.78 3391
14	207.96	238.2
15	263.74	266.76
16	322.46	331.98
17	385.82	376.52
18	426.32	403.12
19	498.14	463.86
20	511.64	458.56
21	512.3	460.78
22	464.48	403.54
23	397.74	339.34
24	338.12	281.8
25	297.86	282.14
26	295.02	278.68
27	292.48	276.52
28	350.56	327.64
29	400.54 5965	372.32 5562
30	534.54	477.12
31	812.84	684.34
32	1470.56	1122.9
33	2540.02	1863.64
34	3690.64	2710.38
35	4753.26	3267.3
36	7842.72	5123.02
37	10209.7	6787.34
38	9004.54	6273.06
39	5482.58	4188.54
40	2628.62	2084.46
41	1263.58	984.26
42	668.1	522.82
43	400.68	319.08
44	234.62	195.04
45	160.3	135.06
46	107.5	90.68
47	96.62 51901	77.08 36906
48	60.36	49.82
	60955	46143

3H counts from FPLC profile.

Author's Signature <i>[Signature]</i>	Date 12-20-96	Read and Understood By <i>[Signature]</i>	Date 1/27/99
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Project Number 565711	Subject TG8 4 wk study	Book Number GDS - 5748
SEARLE		Page 183

TG8 CETP Activity/ 4 weeks on diet



	TG- 1 hr	TG 2 hr	
VLDL	2761	3391	0
% of total	5	7	#DIV/0!
LDL	5965	5562	0
% of total	10	12	#DIV/0!
HDL	51901	36906	0
% of total	85	80	#DIV/0!
Total	60955	46143	0
% transfer	14.32	19.40	#DIV/0!

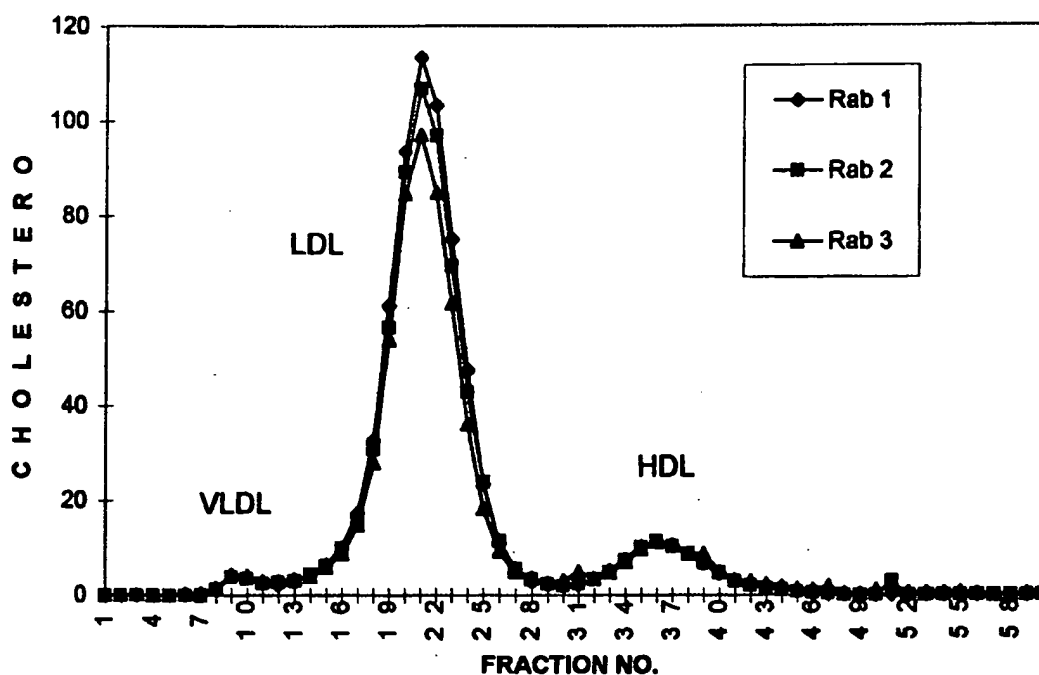
1-10-97 We have decided to change the diet to a 1% chof with cocoa butter to try to increase the lipids. The LDC M VLDL is not high enough to accept any CE being transferred.

Author's Signature <i>Powerlyker</i>	Date 12-20-96	Read and Understood By <i>Rozhan</i>	Date 1/27/99
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Book Number GDS - 5748	Subject FPLC Profile on Rabbit Sera	Project Number 565711
Page 184		SEARLE

1-14-97 Rabbit Sera (obtained from Harriet Kurlander)
and lipoproteins isolated (p.145) and stored -4°C.

Shaved and pooled 6 vials, ~2000 µl, filtered
0.2 µm, injected 500 µl x 3 ^{on 1-27-97} onto Superose 6x2
FPLC.



	RAB 1	RAB 2	RAB 3
VLDL fx 5-13 <i>µg</i>	10.2	8.6	10.4
%	1.4	1.3	1.6
LDL fx 14-29	609.6	576	518.5
%	86	85	81.5
HDL fx 30-47	82	81.4	91.7
%	11.6	12	14.4
^{on 1-27-97} TOTAL COUNTS <i>CHOL</i>	709.4	677.1	636.2

Author's Signature <i>Powerex/Keece</i>	Date <i>1-14-97</i>	Read and Understood By <i>[Signature]</i>	Date <i>1/27/97</i>	Author's <i>[Signature]</i>
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Project Number 565711	Subject Rab. Sera FPLC profile	Book Number GDS - 5748
SEARLE		Page 185

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.001	0.001	0.001	0.034	0.111	0.148	0.039	0.027	0.003	0.002	-0.014	-0.014
B	0.035	0.039	0.003	0.030	0.204	0.073	0.053	0.020	0.003	0.005	-0.013	-0.013
C	0.084	0.087	0.002	0.023	0.374	0.041	0.070	0.017	0.003	0.003	-0.013	-0.013
D	0.162	0.164	0.003	0.021	0.569	0.025	0.075	0.016	0.003	0.003	-0.013	-0.014
E	0.324	0.306	0.003	0.025	0.688	0.021	0.071	0.012	0.003	0.005	-0.013	-0.014
F	0.459	0.482	0.003	0.033	0.627	0.019	0.060	0.010	0.003	0.002	-0.013	-0.014
G	0.577	0.636	0.011	0.046	0.458	0.021	0.048	0.009	0.008	0.002	-0.013	-0.013
H	0.755	0.752	0.017	0.068	0.292	0.029	0.035	0.007	0.003	0.003	-0.013	-0.013

READ DATE:

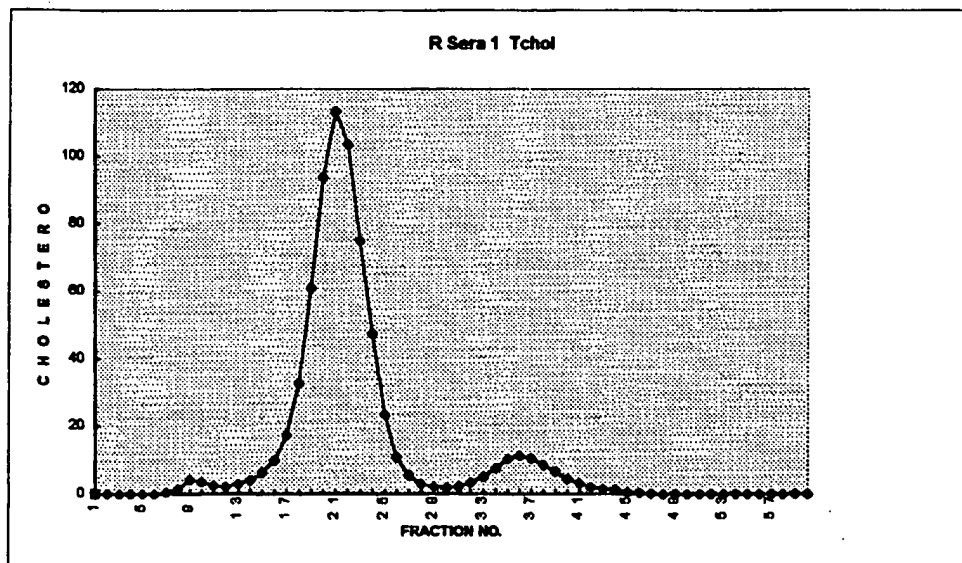
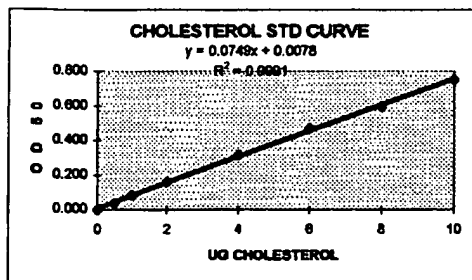
1/14/97

ASSAY NAME:

R Sera 1 Tchol

CHOLESTEROL ASSAY

ug	OD 1	OD 2	MEAN	SD								CALC
STD	OD 1	OD 2	OD	OD								STD
0	0.001	0.001	0.001	0.000	m	b						-0.090
0.5	0.035	0.039	0.037	0.003	0.0749	0.0078	#N/A	#N/A	#N/A	#N/A	#N/A	0.390
1	0.084	0.087	0.086	0.002	0.0009	0.0049	#N/A	#N/A	#N/A	#N/A	#N/A	1.038
2	0.162	0.164	0.163	0.001	0.9991	0.0092	#N/A	#N/A	#N/A	#N/A	#N/A	2.072
4	0.324	0.306	0.315	0.013	#####	6.000	#N/A	#N/A	#N/A	#N/A	#N/A	4.101
6	0.459	0.482	0.471	0.016	0.546	0.001	#N/A	#N/A	#N/A	#N/A	#N/A	6.177
8	0.577	0.616	0.597	0.028								7.858
10	0.755	0.752	0.754	0.002								9.954



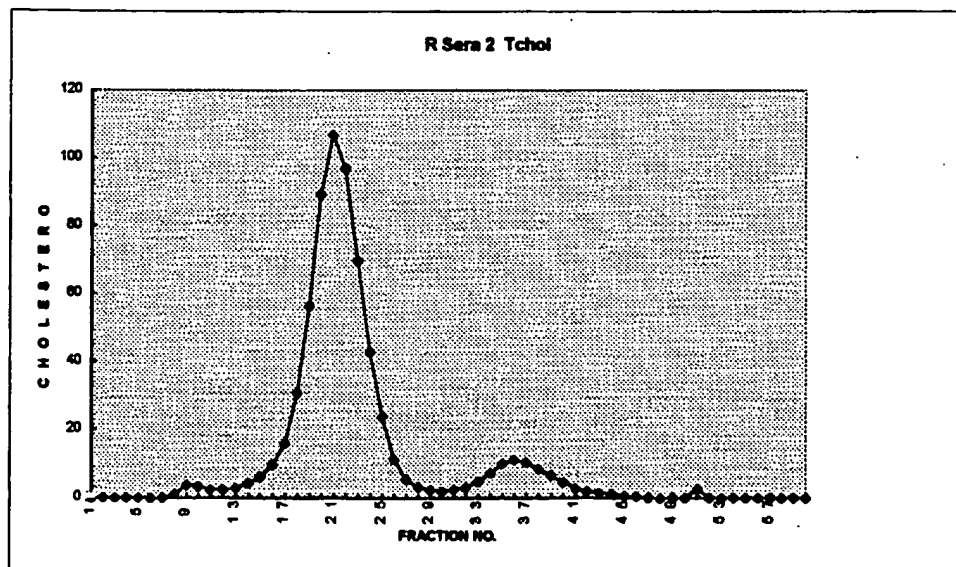
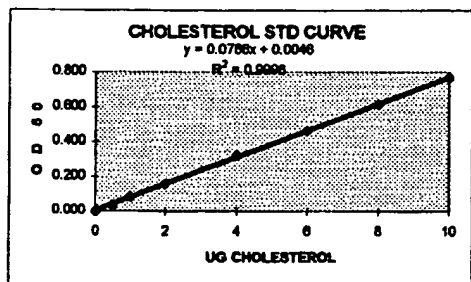
Author's Signature Bwarykepec	Date 1-14-97	Read and Understood by Chs Kohman	Date 1/22/97
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Book Number GDS - 5748	Subject Rab. Sera FPLC Profile, cont	Project Number 565711
Page 186		SEARLE

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.003	0.000	0.000	0.028	0.103	0.151	0.034	0.023	0.002	0.001	-0.018	-0.018
B	0.033	0.037	0.000	0.028	0.183	0.075	0.050	0.017	0.001	0.001	-0.018	-0.016
C	0.084	0.088	0.000	0.020	0.350	0.039	0.087	0.014	0.022	0.001	-0.018	-0.017
D	0.157	0.182	0.000	0.021	0.552	0.025	0.074	0.012	0.001	0.001	-0.015	-0.017
E	0.324	0.319	0.001	0.023	0.660	0.019	0.069	0.009	0.002	0.001	-0.016	-0.014
F	0.466	0.464	0.001	0.031	0.599	0.017	0.058	0.008	0.004	0.000	-0.011	-0.017
G	0.612	0.604	0.005	0.043	0.432	0.020	0.046	0.006	0.002	0.001	-0.016	-0.016
H	0.757	0.780	0.012	0.065	0.267	0.025	0.033	0.004	0.002	0.000	-0.016	-0.016

READ DATE:
1/14/97
ASSAY NAME:
R Sera 2 Tchol

CHOLESTEROL ASSAY											
STD	ug	OD 1	OD 2	MEAN OD	SD OD						CALC STD
0	0.003	0.000	0.002	0.002	0.002	m	b				-0.041
0.5	0.033	0.037	0.035	0.033	0.003	0.0768	0.0048	#N/A	#N/A	#N/A	0.397
1	0.084	0.088	0.085	0.085	0.001	0.0006	0.0032	#N/A	#N/A	#N/A	1.049
2	0.157	0.162	0.160	0.160	0.004	0.9998	0.0060	#N/A	#N/A	#N/A	2.022
4	0.324	0.319	0.322	0.322	0.004	#####	6.000	#N/A	#N/A	#N/A	4.137
6	0.466	0.464	0.465	0.465	0.001	0.571	0.000	#N/A	#N/A	#N/A	6.010
8	0.612	0.616	0.614	0.614	0.003						7.955
10	0.757	0.780	0.769	0.769	0.016						9.972



Author's Signature Beverly Kekec	Date 1-14-97	Read and Understood By [Signature]	Date 1/27/99
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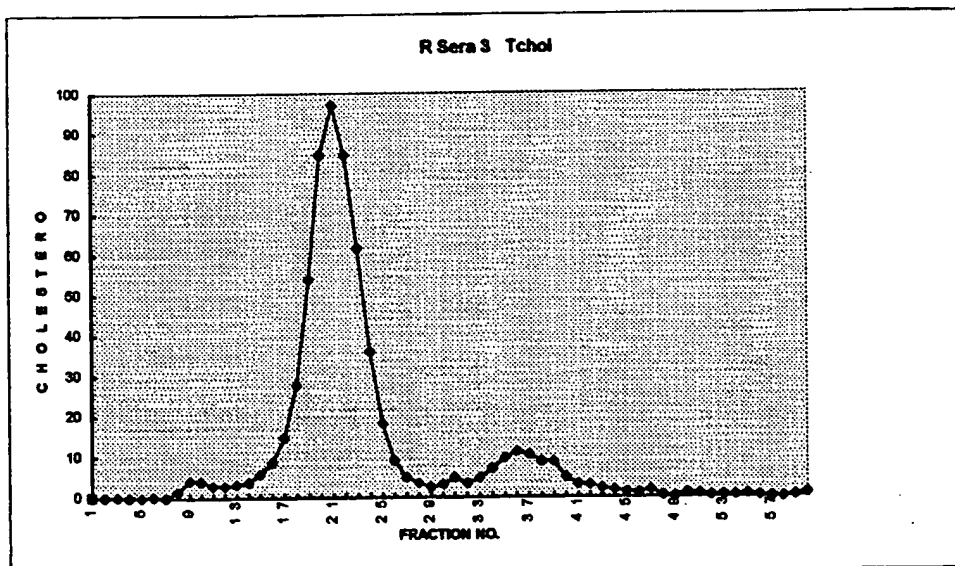
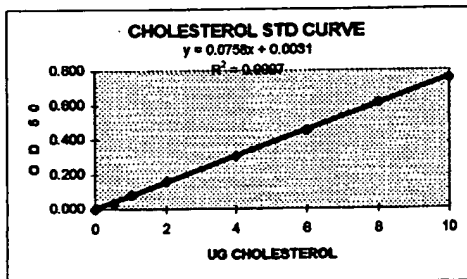
Author

Project Number 565711	Subject Cont.	Book Number GDS - 5748
SEARLE		Page 187

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	0.001	-0.001	0.028	0.083	0.114	0.032	0.022	0.005	0.003	-0.015	-0.015
B	0.031	0.038	0.001	0.028	0.172	0.059	0.046	0.021	0.010	0.003	-0.014	-0.015
C	0.077	0.088	0.004	0.021	0.330	0.033	0.062	0.017	0.008	0.005	-0.014	-0.015
D	0.160	0.183	0.003	0.021	0.517	0.025	0.071	0.014	0.008	0.008	-0.013	-0.015
E	0.304	0.308	0.003	0.023	0.582	0.019	0.087	0.011	0.006	0.009	-0.012	-0.014
F	0.445	0.465	0.005	0.027	0.518	0.022	0.056	0.010	0.006	0.013	-0.013	-0.015
G	0.615	0.609	0.003	0.039	0.377	0.033	0.056	0.014	0.007	0.007	-0.013	-0.016
H	0.759	0.754	0.013	0.056	0.222	0.024	0.033	0.006	0.005	0.003	-0.013	-0.015

READ DATE:
1/14/97
ASSAY NAME:
R Sera 3 Tchol

CHOLESTEROL ASSAY											
ug	OD 1	OD 2	MEAN	SD							
STD	OD	OD	OD	OD	m	b					CALC
0	0.000	0.001	0.001	0.001			#N/A	#N/A	#N/A	#N/A	-0.034
0.5	0.031	0.038	0.035	0.005	0.0758	0.0031	#N/A	#N/A	#N/A	#N/A	0.415
1	0.077	0.088	0.083	0.008	0.0005	0.0029	#N/A	#N/A	#N/A	#N/A	1.048
2	0.160	0.163	0.162	0.002	0.9997	0.0054	#N/A	#N/A	#N/A	#N/A	2.090
4	0.304	0.308	0.306	0.003	#####	6.000	#N/A	#N/A	#N/A	#N/A	3.997
6	0.445	0.465	0.455	0.014	0.558	0.000	#N/A	#N/A	#N/A	#N/A	5.963
8	0.615	0.618	0.616	0.001							8.080
10	0.759	0.754	0.757	0.004							9.941



Author's Signature Beverly Kere	Date 1-14-97	Read and Understood By [Signature]	Date 1/27/97
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Book Number GDS - 5748	Subject LDL 151 Cholesterol	Project Number 565711
Page 188		SEARLE

1	2	3	4	5	6	7	8	9	10	11	12
0.001	0.001	0.147	0.145	0.000	0.002	-0.018	-0.018	-0.018	-0.011	-0.017	-0.017
0.038	0.040	0.139	0.145	0.002	0.005	-0.012	-0.010	-0.018	-0.018	-0.015	-0.017
0.085	0.093	0.210	0.203	0.002	0.005	-0.014	-0.013	-0.015	-0.015	-0.018	-0.018
0.180	0.173	0.212	0.210	0.005	0.007	-0.018	-0.014	-0.018	-0.018	-0.018	-0.017
0.315	0.315	0.287	0.288	0.010	0.010	-0.011	-0.012	-0.009	-0.013	-0.018	-0.017
0.478	0.478	0.286	0.283	0.010	0.006	-0.018	-0.015	-0.018	-0.018	-0.015	-0.017
0.627	0.621	0.359	0.354	0.006	0.005	-0.012	-0.012	-0.011	-0.018	-0.018	-0.017
0.777	0.788	0.362	0.359	0.001	0.005	-0.015	-0.018	-0.018	-0.017	-0.018	-0.014

READ DATE:

1/20/97

ASSAY NAME:

LDL151 TCHOL

CHOLESTEROL ASSAY

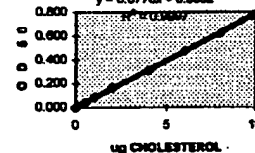
ug STD	OD 1	OD 2	MEAN OD	SD OD								CALC STD
0	0.001	0.001	0.001	0.000	m	b	#N/A	#N/A	#N/A	#N/A	#N/A	-0.054
0.5	0.036	0.040	0.038	0.003	0.0778	0.0052	#N/A	#N/A	#N/A	#N/A	#N/A	0.421
1	0.085	0.093	0.089	0.006	0.0005	0.0027	#N/A	#N/A	#N/A	#N/A	#N/A	1.077
2	0.180	0.173	0.187	0.009	0.9997	0.0051	#N/A	#N/A	#N/A	#N/A	#N/A	2.073
4	0.315	0.315	0.315	0.000	#####	6.000	#N/A	#N/A	#N/A	#N/A	#N/A	3.982
6	0.478	0.478	0.478	0.000	0.588	0.000	#N/A	#N/A	#N/A	#N/A	#N/A	6.052
8	0.627	0.621	0.624	0.004								7.955
10	0.777	0.788	0.783	0.008								9.983

SAMPLE RESULTS (DUPLICATES)

SAMP. NO.	(ml)	OD 1	OD 2	MEAN OD	SD OD	CALC. ug	CALC. ug/ml	DF	mg/dl CMOL
1	0.020	0.147	0.145	0.146	0.001	1.810	90.486	100.000	904.862
2	0.020	0.139	0.145	0.142	0.004	1.758	87.915	100.000	879.149
3	0.030	0.210	0.203	0.207	0.005	2.588	88.252	100.000	882.517
4	0.030	0.212	0.210	0.211	0.001	2.845	88.180	100.000	881.802
5	0.040	0.287	0.288	0.288	0.001	3.629	90.724	100.000	907.235
6	0.040	0.286	0.283	0.285	0.002	3.580	89.759	100.000	897.593
7	0.050	0.359	0.354	0.357	0.004	4.518	90.321	100.000	903.210
8	0.050	0.362	0.359	0.361	0.002	4.587	91.350	100.000	913.495

CHOLESTEROL STD CURVE

$$y = 0.0778x + 0.0052$$



filename : LDL151CH.XLS

Data given to Debbie Howceman.

8939 mg/ml
7.94 mg/ml

Author's Signature <i>Beverly Kene</i>	Date 1-20-97	Read and Understood By <i>[Signature]</i>	Date 1/27/99
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Author's
